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(54) Title: RECOMBINANT PROTEINS DERIVED FROM HGF AND MSP

(57) Abstract

Recombinant proteins deriving from recombination of structural domains deriving from the α subunits of HGF and/or MSP growth factors. The recombinant proteins of the present invention have biological activity, and protect cells from death (apoptosis) induced by chemotherapeutic drugs. These molecules can conveninently be used to prevent or to treat the toxic side effects of chemotherapeutic agents used in cancer therapy.

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RECOMBINANT PROTEINS DERIVED FROM HGF AND MSP

Field of the invention

The present invention relates to recombinant proteins obtained from the combination of structural domains derived from the α subunits of hepatocyte growth factor (HGF) and macrophage stimulating protein (MSP).

In particular, the engineered factors of the invention are obtained by combination of the hairpin loop and kringle domains of the α chains of HGF and/or MSP, so as to obtain a structure having two superdomains with an intervening linker sequence. Moreover, the invention relates to DNA sequences encoding the above mentioned recombinant proteins, to the expression vectors comprising said DNA sequences and to host cells containing said expression vectors. The recombinant proteins of the present invention are biologically active, and their activity can be measured by determination of their ability to induce activation of the Met tyrosine kinase receptor, their "scattering" effect on epithelial cells, and their protective effect against cell death induced by chemotherapic drugs (vide infra). Therefore, these molecules can conveniently be used to prevent or treat the toxic side effects of the chemotherapeutical treatment of tumours, and to reduce iatrogenic cell damage induced by other types of drugs.

Technological background

Hepatocyte Growth Factor (HGF) and Macrophage Stimulating Protein (MSP) are highly related proteins both structurally and

functionally (Fig. 1 and 2). Both these factors are secreted as an inactive precursor, which is processed by specific proteases which recognise a cleavage site inside the molecule, dividing the protein in two subunits. These subunits, named α chain and β chain, are linked by a disulphide bond. Thus, the mature factor is an α-β dimeric protein. Only the mature (dimeric) form of the factor is able to activate its receptor at the surface of the target cells (the Met tyrosine kinase in the case of HGF and the Ron tyrosine kinase in the case of MSP) and therefore to mediate biological responses (Naldini, L. et al., 1992, EMBO J. 11: 4825-4833; Wang, M. et al., 1994, J. Biol. Chem. 269; 3436-3440; Bottaro, D. et al., 1991, Science 25: 802-804; Naldini, L. et al., 1991, EMBO J. 10: 2867-2878; Wang, M. et al., 1994, Science 266: 117-119; Gaudino, G. et al., 1994, EMBO J. 13: 3524-3532).

The α chain of both factors contains a hairpin loop (HL) structure and four domains with a tangle-like structure named kringles (K1-K4; Nakamura T et al., 1989, Nature 342:440-443; Han, S. et al.., 1991, Biochemistry 30: 9768-9780). The precursor also contains a signal sequence (LS) of 31 amino acids (in the case of HGF) or of 18 amino acids (in the case of MSP), removed in rough endoplasmic reticulum, which directs the neoformed peptide to the secretive pathway. The β chain contains a box with a sequence homologous to that typical of serine proteases, but it has no catalytic activity (Nakamura T et al., 1989, Nature 342:440-443; Han, S. et al.., 1991, Biochemistry 30: 9768-9780). Both α and β chains contribute to the binding of the growth factor to the respective receptor (Met for HGF and

Ron for MSP).

HGF and MSP polypeptides are able to induce a variety of biological effects besides cell proliferation. The main biological activities of these molecules are: stimulation of cell division (mitogenesis); stimulation of motility (scattering); induction of polarisation and cell differentiation; induction of tubule formation (branched morphogenesis); increase of cell survival (protection from apoptosis). The tissues that respond to HGF and MSP stimulation are those where cells express the respective Met (HGF) and Ron (MSP) receptors. The most important target tissues of these factors are epithelial cells of different organs, such as liver, kidney, lung, breast, pancreas and stomach, and some cells of the hematopoietic and nervous systems. A detailed review of the biological effects of HGF and MSP in the various tissues can be found in Tamagnone, L. & Comoglio, P., 1997, Cytokine & Growth Factor Re-views, 8: 129-142, Elsevier Science Ltd.; Zarnegar, R. & Michalopoulos, G., 1995, J. Cell Biol. 129: 1177-1180; Medico, E. et al., 1996, Mol. Biol. Cell, 7: 495-504; Banu, N. et al., 1996, J. Immunol. 156: S2933-2940.

In the case of HGF, the hairpin loop and the first two kringles are known to contain the sites of direct interaction with the Met receptor (Lokker NA et al., 1992, EMBO J., 11:2503-2510; Lokker, N. et al., 1994, Protein Engineering 7: 895-903). Two naturally-occurring truncated forms of HGF produced by some cells by alternative splicing have been described. The first one comprises the first kringle (NK1-HGF Cioce, V. et al., 1996, J. Biol. Chem.

271: 13110-13115) whereas the second one spans to the second kringle (NK2-HGF Miyazawa, K. et al., 1991, Eur. J. Biochem. 197: 15-22). NK2-HGF induces cell scattering, but it is not mitogenic as the complete growth factor is (Hartmann, G. et al., 1992, Proc. Natl. Acad. Sci. USA 89: 11574-11578). However, NK2-HGF regains mitogenic activity in the presence of heparin, a glucosaminoglycan that binds HGF through a domain contained in the first kringle and which is likely to induce dimerization of NK2-HGF (Schwall, R. et al., 1996, J. Cell Biol. 133: 709-718). Moreover NK2-HGF, being a partial agonist of Met, behaves as a competitive inhibitor of HGF as far as the mitogenic activity is concerned (Chan, A. et al., 1991, Science 254: 1382-1385). NK1-HGF has also been described to exert partial stimulation of Met and competitive inhibition of HGF mitogenic activity (Cioce, V. et al., 1996, J. Biol. Chem. 271: 13110-13115). Anyway, a truncated factor is endowed with an activity markedly lower than the recombinant factors described in the invention, as shown in example 3.

In the case of MSP, the interaction sites with the Ron receptor are less understood: some preliminary studies suggest a situation opposite of that of HGF, i.e. the β chain directly binds the receptor whereas the α chain would act stabilizing the complex (Wang MH et al., 1997, J. Biol. Chem. 272:16999-17004).

The therapeutical use of molecules such as HGF and MSP is potentially valuable in a wide range of pathologies (Abdulla, S., 1997, Mol. Med. Today 3: 233). Nevertheless, a number of technical

as well as biological complications make the application of these molecules in clinics difficult. First of all, the pleiotropic character of these factors can causes poorly selective biological responses, which involve undesired side effects. For example, the use of HGF to prevent some side effects of the chemotherapeutic drug cisplatin has been proposed (Kawaida K. et al., 1994, Proc. Natl. Acad. Sci. 91:4357-4361). Cancer patients treated with this drug can suffer kidney acute damage due to the cytotoxic action of cisplatin on proximal tubule epithelial cells. HGF is able to protect these cells against programmed death (apoptosis) induced by cisplatin, but at the same time it can induce an undesired proliferation of neoplastic cells. Other problems related to the pharmaceutical use of HGF and MSP are the necessity of their proteolytic activation and their stability, which causes technical problems. The NK1 and NK2 truncated forms of HGF do not require proteolytic activation, but they have a reduced biological activity.

Summary of the invention

The present invention provides recombinant molecules composed of a combination of structural domains derived from the α chains of HGF and/or MSP, which overcome the problems of the prior art molecules described above. The molecules of this invention are composed of two superdomains connected by a linker. Each superdomain is composed of a combination of the HL and K1-K4 domains of the α chain of HGF and/or MSP. These engineered factors induce selective biological responses, do not require

proteolytic activation, are stable and are more active than the truncated forms of HGF described previously.

Detailed disclosure of the invention

The present invention relates to recombinant proteins (which will be hereinafter referred to indifferently as proteins, molecules, engineered or recombinant factors) characterised by a structure that comprises two superdomains, each consisting of a combination of HL and K1-K4 domains derived from the α chain of HGF and/or MSP, linked by a spacer sequence or a linker. In particular, the invention relates to proteins of general formula (I)

$$[A] - B - [C] - (D)_y \qquad (I)$$

in which

[A] corresponds to the sequence $(LS)_m$-HL-K1-$(K2)_n$-$(K3)_o$-$(K4)_p$

wherein (the numbering of the following amino acids refers to the HGF and MSP sequences as reported in Fig. 1 and 2, respectively):

LS is an amino acid sequence corresponding to residues 1-31 of HGF or 1-18 of MSP;

HL is an amino acid sequence derived from the α chain of HGF starting between residues 32-70 and ending between residues 96-127; or it is an amino acid sequence derived from the α chain of MSP starting between residues 19-56 and ending between residues 78-109;

K1 is an amino acid sequence derived from the α chain of HGF starting between residues 97-128 and ending between residues 201-205; or it is an amino acid sequence derived from the α chain of MSP starting between residues 79-110 and ending between residues 186-190;

K2 is an amino acid sequence derived from the α chain of HGF starting between residues 202-206 and ending between residues 283-299; or it is an amino acid sequence derived from the α chain of MSP starting between residues 187-191 and ending between residues 268-282;

K3 is an amino acid sequence derived from the α chain of HGF starting between residues 284-300 and ending between residues 378-385; or it is an amino acid sequence derived from the α chain of MSP starting between residues 269-283 and ending between residues 361-369;

K4 is an amino acid sequence derived from the α chain of HGF starting between residues 379-386 and ending between residues 464-487; or it is an amino acid sequence derived from the α chain of MSP starting between residues 362-370 and ending between residues 448-481;

m, n, o, p can be 0 or 1;

the sum n + o + p is an integer from 1 to 3 or 0, with the proviso that $n \geq o \geq p$;

B is the sequence $[(X)_q Y]_r$, wherein X = Gly and Y = Ser, or Cys, or Met, or Ala;

q is an integer from 2 to 8;

r is an integer from 1 to 9;

[C] corresponds to the sequence HL-K1-$(K2)_s$-$(K3)_t$-$(K4)_u$

wherein HL, K1-K4 are as defined above,

s, t, u are 0 or 1; the sum s + t + u is an integer from 1 to 3 or 0, with the proviso that $s \geq t \geq u$;

D is the sequence W-Z, wherein W is a conventional proteolytic site, Z is any tag sequence useful for the purification and detection of the protein; y

is 0 or 1.

Non-limiting examples of W are consensus sequences for enterokinase protease, thrombin, factor Xa and IgA protease.

Preferred proteins of general formula (I), are those in which: the HL domain is a sequence of HGF α chain ranging from amino acids 32 to 127, or a sequence of MPS α chain ranging from amino acids 19 to 98; the K1 domain is a sequence of HGF α chain ranging from amino acids 128 to 203, or a sequence of MPS α chain ranging from amino acids 99 to 188; the K2 domain is a sequence of HGF α chain ranging from amino acids 204 to 294, or a sequence of MPS α chain ranging from amino acids 189 to 274; the K3 domain is a sequence of HGF α chain ranging from amino acids 286 to 383, or a sequence of MPS α chain ranging from amino acids 275 and 367; the K4 domain is a sequence of HGF α chain ranging from amino acids 384 to 487, or a sequence of MPS α chain ranging from amino acids 368 and 477.

Among the possible combinations of the domains of general formula (I), the following (II) and (III) are preferred, concerning two recombinant factors named Metron Factor-1 and Magic Factor-1, respectively:

LS_{MSP}-HL_{MSP}-$K1_{MSP}$-$K2_{MSP}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (Metron Factor-1) (II)

and

LS_{HGF}-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (Magic Factor-1) (III)

For both molecules, L is a linker sequence $(Gly_4Ser)_3$, D is a tag

sequence Asp_4-Lys-His_6.

For Metron Factor-1, LS_{MSP} is the sequence 1-18 of MSP, HL_{MSP} is the sequence 19-56 of MSP, $K1_{MSP}$ is the sequence 99-188 of MSP, $K2_{MSP}$ is the sequence 189-274 of MSP, HL_{HGF} is the sequence 32-127 of HGF, $K1_{HGF}$ is the sequence 128-203 of HGF, $K2_{HGF}$ is the sequence 204-294 of HGF.

For Magic Factor-1, HL_{HGF}, $K1_{HGF}$, $K2_{HGF}$ are as defined above, LS_{HGF} is the sequence 1-31 of HGF.

The hybrid molecules of the invention are prepared by genetic engineering techniques according to a strategy involving the following steps:

a) construction of DNA encoding the desired protein;

b) insertion of DNA in an expression vector;

c) transformation of a host cell with recombinant DNA (rDNA);

d) culture of the transformed host cell so as to express the recombinant protein;

e) extraction and purification of the produced recombinant protein.

The DNA sequences corresponding to HGF or MSP structural domains can be obtained by synthesis or starting from DNA encoding for the two natural factors. For example, screening of cDNA libraries can be carried out using suitable probes, so as to isolate HGF or MSP cDNA. Alternatively, HGF or MSP cDNA can be obtained by reverse transcription from purified mRNA from suitable cells.

cDNAs coding for the fragments of HGF and MSP β chains can be amplificated by PCR (Mullis, K.B. and Faloona, F.A., 1987, Methods in

Enzymol. 155, 335-350), and the amplification products can be recombined making use of suitable restriction sites, naturally occurring in the factor sequences or artificially introduced in the oligonucleotide sequence used for the amplification.

In greater detail, one of the above mentioned strategies can be the following:

the portions of DNA encoding the LS, HL, K1, K2, K3 and K4 domains are amplificated by PCR from HGF or MSP cDNA and then recombined to obtain the hybrid sequences corresponding to [A] and [C]. Oligonucleotides recognising sequences located at the two ends of the domains to be amplificated are used as primers. Primers are designed so as to contain a sequence allowing recombination between the DNA of a domain and the adjacent one. Said recombination can be carried out by endonuclease cleavage and subsequent ligase reaction, or making use of the recombinant PCR method (Innis, NA et al.., 1990, in PCR Protocols, Academic Press, 177-183).

The sequence encoding the domain B (linker) can be obtained by synthesis of a double chain oligonucleotide, which can be inserted between [A] and [C] using suitable restriction sites.

The resulting three fragments encoding for [A], [B] and [C] are then inserted in the correct sequence in a suitable vector. In this step it can be decided whether to add or not the domain D (tag), obtained by synthesis analogously to domain B, downstream fragment [C].

The recombinant expression vector can contain, in addition to the recombinant construct, a promoter, a ribosome binding site, an initiation

codon, a stop codon, optionally a consensus site for expression enhancers.

The vector can also comprise a selection marker for isolating the host cells containing the DNA construct. Yeast or bacteria plasmids, such as plasmids suitable for Escherichia Coli, can be used as vectors, as well as bacteriophages, viruses, retroviruses, or DNA.

The vectors are cloned preferably in bacterial cells, for example in Escherichia Coli, as described in Sambrook J., 1989, Molecular Cloning, Cold Spring Harbor Laboratory Press, New York, and the colonies can be selected, for example, by hybridisation with radiolabelled oligonucleotide probes; subsequently, the rDNA sequence extracted from the positive colonies is determined by known methods.

The vector with the recombinant construct can be introduced in the host cell according to the competent cell method, the protoplast method, the calcium phosphate method, the DEAE-dextran method, the electric impulses method, the in vitro packaging method, the viral vector method, the micro-injection method, or other suitable techniques.

Host cells can be prokaryotic or eukaryotic, such as bacteria, yeasts or mammal cells, and they will be such as to effectively produce the recombinant protein.

After transformation, cells are grown in a suitable medium, which can be for example MEM, DMEM or RPMI 1640 in the case of mammal host cells.

The recombinant protein is secreted in the culture medium from which it can be recovered and purified with different methods, such as mass exclusion, absorption, affinity chromatography, salting-out, precipitation, dialysis, ultrafiltration.

A simple, rapid system for the production of the molecules of the invention is, for example, transient expression in mammal cells.

Accordingly, the plasmid containing the recombinant DNA fragment, for example PMT2 (Sambrook, J. et al., 1989, Molecular Cloning, Cold Spring Harbor Laboratory Press), is transfected in suitable recipient cells, such as Cos7 (Sambrook, J. et al., supra) by the calcium phosphate technique or other equivalent techniques. Some days after transfection, the conditioned medium of the transfected cells is collected, cleared by centrifugation and analysed for its content in factor. For this analysis, antibodies directed against HGF or MSP, or against any tag sequence, can be used: the supernatant is immunoprecipitated and then analysed by western blot with the same antibody. The supernatant containing the recombinant factor can also be used directly for biochemical and biological tests. The protein can be purified, for example, using a poly-histidine tag sequence, by absorption on a nickel resin column and subsequent elution with imidazole.

The biochemical properties of the recombinant factors of the invention were tested in connection with their ability to activate Met and Ron receptors.

Sub-micromolar concentrations of the factors have proved to induce phosphorylation in Met tyrosine in human epithelial cells A549, whereas

they do not induce phosphorylation above basal values in cells expressing Ron. On the whole, the tests proved that the first two kringles of HGF maintain their ability to interact and to activate Met tyrosine kinase receptor, whereas the corresponding first two kringles of MSP are not sufficient for modulating the catalytic activity of the Ron receptor. However, the interaction with Ron, although at low affinity, can contribute to the recruitment of the factor at the cell surface, playing a similar role to low affinity receptors (of mature glycoprotein) which recruit the HGF intact molecule through the heparin-binding domain.

The molecules of the invention have a marked biological activity, measured by the scattering tests, and a protecting activity against cell apoptosis induced by cisplatin or etoposide.

In particular, the supernatant containing the recombinant factor has been found to promote scattering of epithelial cells of various nature even at nanomolar concentrations. In these tests, kidney epithelial cells (MDCK) or hepatocyte precursors (MLP29) were used.

In an in vitro experimental system, in which DNA fragmentation typical of apoptotic cells is evaluated by the TUNEL method (Gavrieli, Y. et al., 1992, J. Cell. Biol. 117, 493-501), the recombinant factors protect against apoptosis induced by chemotherapeutic drugs at levels comparable with HGF and remarkably higher than MSP. The engineered molecules proved to be active on human primary epithelial cells from proximal tubule (PTECs), on an immortalised PTECs line (Loc) and on the already cited murine hepatocytes MLP29.

Among the applications of the recombinant molecules of the

invention, the following can be cited:

- prevention of myelotoxicity; in particular they can be used for the expansion of marrow precursors, to increase proliferation of the hematopoietic precursors or to stimulate their entry in circle;
- prevention of liver and kidney toxicity, and of mucositis following antineoplastic treatments; in particular the recombinant factors can be used to prevent toxicity (apoptosis) on differentiated cell elements of liver, kidney and mucosa of the gastroenteral tract, and to stimulate staminal elements of cutis and mucosas to allow the regeneration of germinative layers;
- prevention of chemotherapeutic neurotoxicity.

In general, the proteins of the invention provide the following advantages, compared with the parent molecules HGF and MSP:

- they are smaller molecules with a more compact structure;
- they are more stable and are produced in higher amounts;
- they require no endoproteolytic cleavage for activation, which transforms the HGF and MSP precursors into the respective active forms;
- they can be engineered in combinations of different functional domains, thereby modulating the biological effects, increasing the favourable ones and reducing those undesired (for example, protection from apoptosis versus cell proliferation).

The invention has to be considered also directed at amino acid and nucleotide sequences referred to formula (I), having modifications which can, for example, derive from degeneration of genetic code, without

therefore modifying the amino acid sequence, or from the deletion, substitution, insertion, inversion or addition of nucleotides and/or bases according to all the possible methods known in the art.

Furthermore, the invention relates to the expression vectors comprising a sequence encoding for a protein of general formula (I), which can be plasmids, bacteriophages, viruses, retroviruses, or others, and to host cells containing said expression vectors.

Finally, the invention relates to the use of the recombinant proteins as therapeutical agents, and to pharmaceutical compositions containing an effective amount of the recombinant proteins together with pharmacologically acceptable excipients.

Description of the Figures

(In the following legends, -His located after the name of the parent factors, truncated or recombinant, or of the plasmids, means that the respective sequences contain a poly-histidine tag).

Figure 1:

a) Nucleotide and amino acid sequence of human HGF (Gene Bank # M73239; Weidner, K.M., et al., 1991, Proc. Acad. Sci. USA, 88:7001-7005). In contrast to the cited reference, in the numbering used herein, nucleotide No. 1 is the first base of the initiation codon (the A of the first ATG). The first amino acid is the corresponding methionine. The cDNA untranslated regions at 5' and 3' are neither represented nor considered in the numbering.

b) Nucleotide and amino acid sequence of human MSP (Gene Bank # L11924; Yoshimura, T., et al., 1993, J. Biol. Chem., 268:15461-

15468). In contrast to the cited reference, in the numbering used herein nucleotide No. 1 is the first base of the initiation codon (the A of the first ATG). The first amino acid is the corresponding methionine. The cDNA untranslated regions at 5' and 3' are neither represented nor considered in the numbering.

Figure 2:

a) Molecular structure of Metron Factor-1. The leader sequence is removed from the cells used for the production before secretion and is therefore absent in the mature molecule. The poly-histidine tag can be removed by digestion with the protease enterokinase.

b) Nucleotide and amino acid sequence of Metron-Factor-1. The nucleotide sequence starts with the EcoRI site and terminates with the SalI site (first six bases and last six bases, respectively). The initiation codon (ATG) and the stop codon (TAG) are underlined.

Figure 3:

a) Molecular structure of Magic Factor-1. The leader sequence is removed from the cells used for the production before secretion and is therefore absent in the mature molecule. Poly-histidine tag can be removed by digestion with the protease enterokinase.

b) Nucleotide and amino acid sequence of Magic Factor-1. The nucleotide sequence starts with the SalI site (first six bases and last six bases, respectively). The initiation codon (ATG) and the stop codon (TAG) are underlined.

Figure 4:

Production of Metron-F-1 by transient transfection of mammal

cells. The conditioned supernatants from BOSC cells transfected with the control plasmid (CTRL) or with pRK7-Metron F-1-His were immunoprecipitated with an anti-MSP polyclonal antibody and detected by western blot with the same antibody.

Figure 5:

Quantitation of the recombinant proteins by western blot. (A) The proteins were absorbed on Sepharose-A-heparin beads and detected with an anti-poly-histidine monoclonal antibody. (B) The proteins were immunoprecipitated with an anti-MSP polyclonal antibody and detected with an anti-poly-histidine monoclonal antibody.

Figure 6:

Scattering test carried out on kidney epithelial cells (MDCK) using the recombinant proteins prepared by transient transfection. The protein content was quantified by western blot (see Fig. 5). (A) non-stimulated cells; (B) cells stimulated with control supernatant; (C) cells stimulated with HGF-His; (D) cells stimulated with NK2-HGF-His; (E) cells stimulated with Metron Factor-1; (F) cells stimulated with Magic Factor-1.

Figure 7:

Activation (phosphorylation) of Met receptor by the hybrid factor Metron Factor-1. Human epithelial cells (A549) were stimulated with supernatants conditioned from BOSC cells transfected with the control plasmid (CTRL) or with pRK7-Metron-F-1-His (METRON F-1) at the indicated dilutions. Cell lysates from the stimulated cells were immunoprecipitated with an anti-Met monoclonal antibody and detected by western blot with an anti-phosphotyrosine monoclonal antibody.

Figure 8:

Protective effect of Metron-F-1 against acute renal failure induced by $HgCl_2$ in vivo. Balb-c mice were injected i.v. with Metron-F-1 or vehicle at 0.5 h before and 6, 12, 24, 36 and 48 h after $HgCl_2$ i.v. administration. BUN and histological evaluation of renal necrosis were measured at 72 h.

Data expressed as mean + e.s. of 7 animals/group (BUN) or 3 animals/group (histology).

The following examples illustrate in greater detail the invention.

Example 1a: Preparation of the recombinant construct encoding Metron Factor-1

HGF cDNA was obtained by the RT-PCR technique (Reverse Transcriptase PCR; in: Innis, M. A., et al.., 1990, PCR Protocols, Academic Press, 21-27) from a human lung fibroblast cell line (MRC5; Naldini, L. et al., 1991, EMBO J. 10: 2867-2878). MSP cDNA was obtained with the same technique from human liver (Gaudino, G., et al., 1994, EMBO J. 13: 3524-3532).

The fragment corresponding to MSP LS-HL-K1-K2 was amplified by PCR using MSP cDNA as template and the following oligonucleotides as primers:

P1 (sense)

5' CGCGCGGAATTCCACCATGGGGTGGCTCCCACTCCT 3'

P2 (antisense)

5' CGCGCGCTCGAGGCGGGGCTGTGCCTCGGACCCGCA 3'

in which the underlined palindromic sequences are the restriction sites for

the enzymes EcoRI (oligonucleotide P1) and XhoI (oligonucleotide P2). The PCR product was digested with the restriction enzymes EcoRI and XhoI and then purified by electrophoresis on agarose gel.

The fragment corresponding to HL-K1-K2 of HGF was amplified by PCR using HGF cDNA as template and the following oligonucleotides as primers:

P3 (sense)

5' CGCGCGTCTAGAGGGACAAAGGAAAAGAAGAAATAC 3'

P4 (antisense)

5' CGCGCGAAGCTTTGTCAGCGCATGTTTTAATTGCAC 3'

in which the underlined palindromic sequences are the restriction sites for the enzymes XbaI (oligonucleotide P3) and HindIII (oligonucleotide P4). The PCR product was digested with the restriction enzymes XbaI and HindIII and then purified by electrophoresis on agarose gel.

For the linker sequence, the following partially complementary oligonucleotides were synthesised, and were subsequently annealed to obtain a double strand DNA fragment with sticky ends:

P5 (sense)

5'TCGAGGGCGGTGGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCT 3'

P6 (antisense)

5'CTAGAGAACCGCCACCGCCGGAGCCACCGCCACCAGAACCGCCACCGCCC 3'

in which the underlined bases are the sequences compatible with the restriction sites for the enzymes XhoI (oligonucleotide P5) and XbaI (oligonucleotide P6).

The resulting three DNA fragments were subcloned in the EcoRI-

HindIII sites of the expression vector pRK7 (Gaudino, G., et al., 1994, EMBO J. 13: 3524-3532), to obtain the recombinant plasmid pRK7-Metron-F-1, containing all the components of Metron Factor-1 except the tag sequence.

For the insertion of the tag sequence, the following partially complementary oligonucleotides were synthesised, and were subsequently annealed to obtain a double strand DNA fragment with sticky ends:

P7 (sense)

5'AGCTGACGACGACGACAAACACCACCACCACCACCACCACTAGGGTCGAC3'

P8 (antisense)

5'AGCTGTCGACCCTAGTGGTGGTGGTGGTGGTGTTTGTCGTCGTCGTC3'

in which the underlined bases are compatible with the HindIII restriction site and the boxed palindromic sequences are the consensus sequences for the enzyme SalI. The resulting double strand DNA fragment was inserted in the restriction site HindIII of the recombinant plasmid obtained at the previous step (destroying the HindIII site and creating the SalI site), to obtain the plasmid pRK7-Metron-F-1-His.

Example 1b: Production of Metron Factor-1

The expression vector pRK7 contains a promoter of human cytomegalovirus immediate-early gene (CMV) and an episomal replication origin site of the DNA virus SV40. Therefore, this plasmid is particularly suitable for the expression of proteins in cells expressing the large T antigen of the virus SV40, such as kidney epithelial BOSC cells (Sambrook, J. et al.., 1989, Molecular Cloning, Cold Spring Harbor Laboratory Press). Metron Factor-1 can then be produced by transient

transfection of plasmid pRK7-Metron F-1-His in BOSC cells.

For transfection, 10^6 cells are seeded at day 0 in a 100 mm plate in 90% Dulbecco's Modified Eagle Medium (DMEM)-10% bovine calf serum (10 ml/plate). At day 1, cells are transfected with 10 µg/plate of pRK7-Metron-F-1-His by lipofection, using the protocol provided by the lipofectin producer (Gibco-BRL). At day 2, the DNA-containing medium is substituted by fresh medium with low content in serum (99.5% DMEM-0.5% bovine calf serum). At day 4 (48 hours after the end of the transfection), the medium is collected, cleared by centrifugation, and analysed for its content in Metron Factor-1.

This analysis can be carried out in different ways. For example, the recombinant protein present in the cleared supernatant can be immunoprecipitated with an anti-MSP antibody and then detected by western blot with the same antibody (Fig. 4). In the example shown in figure 4, 500 µl of supernatant (cleared by centrifugation, buffered in 25 mM HEPES and added with a protease inhibitors cocktail) were immunoprecipitated (2 hours at 4° C) with 20 µl of Sepharose-A beads (Pharmacia) covalently conjugated with 2 µl of anti-MSP polyclonal antibody. The beads pellet was washed 3 times with 500 µl of washing buffer (20 mM HEPES pH 7.4; 150 mM NaCl; 0.1% Triton X-100; 10% glycerol) and heated at 90° C for 2 minutes in 100 µl of Laemmli buffer. Eluted proteins were separated by SDS-PAGE on 8% BIS-acrylamide gel, transferred onto membrane (Hybond-C; Amersham) and analysed by western blot. For this analysis, the same rabbit serum used for immunoprecipitation was employed as primary antibody with a 1:1000

dilution and protein A conjugated with peroxidase (Amersham) was used as secondary antibody. Protein A was detected by ECL (Amersham) following the protocol provided by the producer.

Alternatively, the recombinant protein can be partially purified by adsorption on Sepharose-A beads conjugated with heparin and subsequent analysis by western blot using antibodies directed to poly-histidine tag (Fig. 5).

In the example shown in figure 5, the Sepharose-A-heparin beads (20 µl; Pierce) were incubated (4 hours at 4° C) with 500 µl of supernatant (cleared by centrifugation, buffered in 25 mM HEPES and added with a protease inhibitors cocktail) in the presence of 500 mM NaCl, washed with suitable buffer (500 mM NaCl; 20 mM HEPES pH 7.4; 0.1% Triton X-100; 10% glycerol) and heated at 90° C for 2 minutes in 100 µl Laemmli buffer. Eluted proteins were separated by SDS-PAGE on 8% bis-acrylamide gel, transferred onto membrane (Hybond-C; Amersham) and analysed by western blot. For this analysis, a mouse monoclonal antibody to poly-histidine (Invitrogen) diluted 1:5000 was used as primary antibody and an anti-mouse IgG ovine antibody conjugated with peroxidase (Amersham) was used as secondary antibody. The secondary antibody was detected by ECL (Amersham) following the protocol provided by the producer.

The procedure of adsorption on heparin beads can also be used as protocol for the semi-purification of the recombinant protein. Furthermore, the molecule can additionally be purified making use of the poly-histidine affinity to heavy metals such as nickel. The protein containing poly-

histidine tag can be adsorbed on a nickel resin column (Invitrogen) and subsequently eluted with imidazole (the detailed protocol is provided by the manufacturer).

Example 1c: METRON-F-1 production in insect cells

The cDNA encoding for Metron-F1 was subcloned in a suitable expression vector (p-FASTBAC) to generate a recombinant plasmid containing the Metron-F1 gene (p-FASTBAC-Metron). A competent E. Coli strain (DH10 Bac) was transformed with p-FASTBAC-Metron to generate BACMID DNA. The DNA of positive colonies was isolated and checked by PCR to show the correct integration of the expression vector. Subsequently, the DNA from three clones was transfected into Sf9 insect cells with CellFECTIN reagent to produce virus particles. Virus titer was tested by a plaque assay. Single plaques were isolated and used for further propagation of the baculovirus. Viral stock was subsequently expanded in insect cells to scale up METRON-F-1 production. To verify protein expression, insect cells were infected with a multiplicity of infection (MOI) of 1 in a small-scale reactor. Samples of supernatants were analysed by SDS-PAGE followed by western blotting.

To produce amounts adequate for in vivo testing, insect cells were propagated in a 2.5-Liter stirred tank bioreactor. Cells were grown to a cell density of 1.106 ml^{-1} before they were infected with a MOI of 1. Cell suspension was harvested 3 days post infection. The supernatant containing the recombinant protein was separated by centrifugation. The presence of Metron F-1 in the supernatant was proved by SDS-PAGE followed by western blotting. Metron F-1 was pre-purified by a dual step

affinity chromatography on heparin sepharose (heparin-Hi Trap, Pharmacia) at 6° C. For in vivo testing or for further purification steps, the eluted fractions containing Metron F-1 were desalted by Sephadex G-25 chromatography (PD-10 or HiPrep 26/10, Pharmacia). Metron F-1 was further purified by chromatography on HisTrap columns (Pharmacia) and eluted by an imidazole gradient (0-0.5 M) using either a low-pressure system (Econo System, BIO-RAD) or an FPLC system (Pharmacia). Metron F-1 was eluted at an imidazole concentration of about 0.15 M. For in vivo testing, the eluted fractions containing Metron F-1 were freed of imidazole by Sephadex G-25 chromatography as already described, using the buffer to be used for animal treatment.

Example 2a: Preparation of the recombinant construct encoding for Magic Factor-1

HGF cDNA and the plasmid pRK7-Metron-F-1-His described above were used as starting DNA. The fragment corresponding to LS-HL-K1-K2 of HGF was amplificated by PCR using HGF cDNA as template and the following oligonucleotides as primers:

P9 (sense)

5' CGCGCGGGATCCGCCAGCCCGTCCAGCAGCACCATG 3'

P10 (antisense)

5' CGCGCGAAGCTTTGTCAGCGCATGTTTTAATTGCAC 3'

in which the underlined palindromic sequences are the restriction sites for the enzymes BamHI (oligonucleotide P9) and HindIII (oligonucleotide P10). The PCR product was digested with the restriction enzymes BamHI and HindIII and then purified by electrophoresis on agarose gel.

For the linker, the following partially complementary oligonucleotides were synthesized, and subsequently annealed to obtain a double strand DNA fragment with sticky ends:

P11 (sense)

5'AGCTTCGGGCGGTGGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCT3'

P12 (antisense)

5'CTAGAGAACCGCCACCGCCGGAGCCACCGCCACCAGAACCGCCACCGCCCGA3'

in which the underlined bases are the sequences compatible with the restriction sites for the enzymes HindIII (oligonucleotide P11) and XbaI (oligonucleotide P12). The fragment resulting by PCR and the double strand linker sequence were inserted in the plasmid pRK7-Metron-F-1-His in place of the fragment EcoRI-XbaI by means of an EcoRI-BamHI adapter, to obtain the plasmid pRK7-Magic-F-1-His.

Example 2b: Production of Magic Factor-1

Magic Factor-1 is produced on a small scale by transient transfection of BOSC cells analogously to what described for Metron Factor-1. Semi-purification is performed by adsorption on Sepharose-A beads conjugated with heparin followed by Western blot analysis using anti-poly-histidine antibodies (Fig. 5).

Example 3: Biological activity (scattering) on epithelial cells.

The biological activity of recombinant HGF, NK2-HGF, Metron Factor-1 and Magic Factor-1 was tested by a "scatter" assay on MDCK epithelial cells. For this functional test, cells are plated at day 0 in 96-well plates (10^3 cells/well) in 90% DMEM - 10% bovine calf serum. At day 1 the medium is substituted with fresh medium buffered with 50 mM

HEPES pH 7.4 and the supernatant containing the recombinant protein is added at different dilutions. At day 2 cells are washed with DPBS (Dulbecco's Phosphate Buffered Saline), fixed in 11% glutaraldehyde, stained with a Crystal-Violet solution and analysed by microscopy. The scattering activity is evaluated observing the morphology of the colonies, which are clustered in the negative control (non-stimulated cells or stimulated with supernatant containing no factors) whereas they are dispersed in the positive control (HGF-His). The morphology of the cells themselves also varies upon stimulation: in fact, as it can be observed in Fig. 6, cells stimulated with HGF-His and Metron Factor-1 have a more oblong, spindle-shaped form, characterised by protrusions of the cell membrane called pseudopodes. These morphological variations are the consequence of factor-induced activation of a genetic program involving the modification of a series of cellular parameters, such as digestion of cell matrix by specific proteases and increase in motility.

The Table summarises the results of different tests, obtained with factors HGF, NK2-HGF, Metron Factor-1 and Magic Factor-1 on MDCK cells. The scattering units reported indicate the maximum dilution of the conditioned supernatant containing the factor, at which motogenic activity could be observed. Values are normalised for the protein content determined by western blotting as described above (see Fig. 5). These data indicate that the hybrid factors Metron Factor-1 and Magic Factor-1 have a scattering activity approximately three magnitudes higher than that of the NK2-HGF-His truncated form and one magnitude higher than that of HGF-His parental factor.

	HGF-his	NK2-his	Metron F-1	Magic Factor-1
Scatter units	900 ± 29	6 ± 5	5500 ± 1532	7600 ± 150

Table. Scattering activity of factors HGF-His, NK2-HGF-His Metron Factor-1 measured on kidney epithelial cells (MDCK). The scattering units reported indicate the maximum dilution of the conditioned supernatant containing the factor, at which a motogenic activity can be observed. Values are normalised for the protein content determined by western blotting.

Example 4a: Test for the evaluation of protection against programmed cell death (apoptosis).

One of the most characterised side effect of the chemotherapeutic drug cisplatin is the induction of programmed cell death (apoptosis) of epithelial cells of the proximal tubule, which leads to acute renal failure (ARF). Thus, a factor that protects against cisplatin-induced cytotoxicity is highly desirable. An in vitro functional test has been used, which allows to evaluate the percentage of cisplatin-treated apoptotic cells in the presence or in the absence of a survival factor. This system utilises a cell line (LOC) derived from epithelial cells of human kidney proximal tubule, immortalised by ectopic expression of SV40 large T antigen. For the functional test, cells are plated at day 0 in 96-well plates (10^3 cells/well) in 90% DMEM - 10% bovine calf serum. At day 1, the medium is substituted with medium containing 0.5% bovine calf serum buffered with 50 mM HEPES pH 7.4, which is added with different dilutions of the supernatant containing the recombinant factor. Cells are pre-incubated with these

factors for 6 hours, and then further incubated in the presence of 10 μg/ml cisplatin. At day 2, cells are washed with DPBS and the percentage of apoptotic cells is evaluated by the TUNEL technique (Boehringer Mannheim). The same kind of tests can be performed using primary cultures of human epithelial cells of kidney proximal tubule (PTEC). These tests proved that Metron Factor-1 and Magic Factor-1 have protecting activity against cisplatin-induced programmed cell death.

Example 4b: Protection against cisplatin-induced cytotoxicity by transient gene delivery of Metron Factor-1 and Magic Factor-1

The protective effect of Metron F-1 and Magic F-1 against cisplatin-induced cytotoxicity was further demonstrated by a transient gene delivery approach. Simian kidney epithelial cells (COS) were transfected with a control empty vector, an expression vector for Metron F-1, or an expression vector for Magic F-1. Following transfection, cells were treated for 16 hours with cisplatin (20 μg/ml) and the percentage of surviving cells in each transfection was determined. Cisplatin treatment was calibrated to cause the death of approximately 20% of the cells in the negative control. Ectopic expression of Metron F-1 or Magic F-1 increased the survival rate to about 92.3% and 94.0%, respectively.

Example 5: Activation of the Met receptor by Metron Factor-1 and Magic Factor-1

The ability of Metron Factor-1 and Magic Factor-1 to activate the Met receptor was tested by analysing the ability of the recombinant factors to induce tyrosine phosphorylation of Met in human epithelial cells (A549). For this analysis, A549 cells at 90% confluence in a 100 mm petri

dish were stimulated for 10 minutes with 1 ml of conditioned supernatant containing Metron Factor-1, Magic Factor-1 or no factor (as negative control) diluted 1:2.5 or 1:10 in DMEM. After stimulation, cells were washed in ice with PBS, lysated in 200 µl of lysis solution (1% Triton X-100, 5 mM EDTA, 150 mM NaCl, 20 mM Tris-HCl pH 7.4), added with a cocktail of protease inhibitors, immunoprecipitated for 2 hours at 4° C with 10 µl of Sepharose-A beads covalently conjugated with an anti-Met monoclonal antibody (Naldini, L. et al., 1991, EMBO J. 10: 2867-2878), washed 3 times in the same lysis solution, and heated at 90°C for 2 minutes to elute the absorbed proteins. These were separated by SDS-PAGE on a 8% BIS-acrylamide gel, transferred onto a membrane (Hybond-C; Amersham) and analysed by western blot. A mouse monoclonal antibody against phosphotyrosine (UBI) diluted 1:10000 was used as primary antibody and an anti-mouse IgG ovine antibody conjugated with peroxidase (Amersham) was used as secondary antibody. The secondary antibody was detected by ECL (Amersham) following the protocol provided by the manufacturer. This analysis revealed that Metron F-1 and Magic F-1 potently activate the Met receptor (Fig. 7).

Example 6: Protection against chemotherapy-induced renal failure by Metron Factor-1 in vivo

Metron-F-1 was tested in a model of nephrotoxicity in Balb-c mice. The method used was substantially as described (Kawaida K et al., 1994, Hepatocyte growth factor prevents acute renal failure and accelerates renal regeneration in mice, Proc. Natl. Acad. Sci. 91:4357-4361). Briefly, renal failure was induced in male Balb-c mice weighing 20-25 g by an i.v.

injection of 7.5 mg/kg of $HgCl_2$ (7 animals/group). Renal damage was assessed by analysis of Blood Urea Nitrogen (BUN) and by histological evaluation, 72 h after $HgCl_2$ injection. Metron-F-1 was dissolved in 0.2 M NaCl, containing 0.01% Tween 80 and 0.25% human serum albumin and administered i.v. (100 µg/kg in a posological volume of 6.6 ml/kg) 0.5 h before and 6, 12, 24, 36 and 48 h after $HgCl_2$ injection. Controls animals were treated with the same amount of vehicle according to the same scheme.

Metron-F-1 significantly prevented the onset of acute renal failure induced by $HgCl_2$, evaluated in terms of BUN (figure 8). BUN values were closely paralleled by the histological findings, scored by an independent investigator.

In the following sequence listing:

SEQ. ID. NO. 1: Magic F-1 DNA coding sequence;

SEQ. ID. NO. 2: Magic F-1 amino acid sequence;

SEQ. ID. NO. 3: Metron F-1 DNA coding sequence;

SEQ. ID. NO. 4: Metron F-1 amino acid sequence.

CLAIMS

1. Recombinant proteins comprising two superdomains, separated by a spacer sequence (linker), obtained combining the HL and K1-K4 domains of HGF and MSP α chains.

2. Recombinant proteins as claimed in claim 1, of general formula (I):

[A] - B - [C] - (D)y (I)

in which

[A] corresponds to the sequence $(LS)_m$-HL-K1-$(K2)_n$-$(K3)_o$-$(K4)_p$

wherein (the numbering of the following amino acids refers to the HGF and MSP sequences as reported in Fig. 1 and 2, respectively):

LS is an amino acid sequence corresponding to residues 1-31 of HGF or 1-18 of MSP;

HL is an amino acid sequence derived from the α chain of HGF starting between residues 32-70 and ending between residues 96-127; or it is an amino acid sequence derived from the α chain of MSP starting between residues 19-56 and ending between residues 78-109;

K1 is an amino acid sequence derived from the α chain of HGF starting between residues 97-128 and ending between residues 201-205; or it is an amino acid sequence derived from the α chain of MSP starting between residues 79-110 and ending between residues 186-190;

K2 is an amino acid sequence derived from the α chain of HGF starting between residues 202-206 and ending between residues 283-299; or it is an amino acid sequence derived from the α chain of MSP starting between residues 187-191 and ending between residues 268-282;

K3 is an amino acid sequence derived from the α chain of HGF starting between residues 284-300 and ending between residues 378-385; or it is an amino acid sequence derived from the α chain of MSP starting between residues 269-283 and ending between residues 361-369;

K4 is an amino acid sequence derived from the α chain of HGF starting between residues 379-386 and ending between residues 464-487; or it is an amino acid sequence derived from the α chain of MSP starting between residues 362-370 and ending between residues 448-481;

m, n, o, p are 0 or 1;

the sum n + o + p is an integer from 1 to 3 or 0, with the proviso that $n \geq o \geq p$;

B is the sequence $[(X)_q\ Y]_r$, wherein X = Gly and Y = Ser, or Cys, or Met, or Ala;

q is an integer from 2 to 8;

r is an integer from 1 to 9;

[C] corresponds to the sequence HL-K1-$(K2)_s$-$(K3)_t$-$(K4)_u$

wherein HL, K1-K4 are as defined above,

s, t, u are 0 or 1; the sum s + t + u is an integer from 1 to 3 or 0, with the proviso that $s \geq t \geq u$;

D is the sequence W-Z, wherein W is a conventional proteolytic site, Z is any tag sequence useful for the purification and detection of the protein; y is 0 or 1.

3. Recombinant proteins according to claims 1-2, in which the HL domain is a sequence of HGF α chain ranging from amino acids 32 to 127, or a sequence of MPS α chain ranging from amino acids 19 to 98; the K1

domain is a sequence of HGF α chain ranging from amino acids 128 to 203, or a sequence of MPS α chain ranging from amino acids 99 to 188; the K2 domain is a sequence of HGF α chain ranging from amino acids 204 to 294, or a sequence of MPS α chain ranging from amino acids 189 to 274; the K3 domain is a sequence of HGF α chain ranging from amino acids 286 to 383, or a sequence of MPS α chain ranging from amino acids 275 to 367; the K4 domain is a sequence of HGF α chain ranging from amino acids 384 to 487, or a sequence of MPS α chain ranging from amino acids 368 to 477.

4. Recombinant proteins according to claims 1-3 of formula (II):

LS_{MSP}-HL_{MSP}-$K1_{MSP}$-$K2_{MSP}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (II)

in which LS_{MSP} is the sequence 1-18 of MSP, HL_{MSP} is the sequence 19-56 of MSP, $K1_{MSP}$ is the sequence 99-188 of MSP, $K2_{MSP}$ is the sequence 189-274 of MSP, HL_{HGF} is the sequence 32-127 of HGF, $K1_{HGF}$ is the sequence 128-203 of HGF, $K2_{HGF}$ is the sequence 204-294 of HGF, L is the sequence $(Gly_4Ser)_3$, D is the sequence Asp_4-Lys-His_6.

5. Recombinant proteins according to claims 1-3 of formula (III):

LS_{HGF}-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-L-HL_{HGF}-$K1_{HGF}$-$K2_{HGF}$-D (III)

in which HL_{HGF}, $K1_{HGF}$, $K2_{HGF}$, L and D are as defined in claim 4, LS_{HGF} is the sequence 1-31 of HGF.

6. Nucleotide sequences encoding for the recombinant proteins of claims 1-5.

7. Expression vectors comprising the nucleotide sequences of claim 6.

8. Prokaryotic or eukaryotic host cell transformed with the expression vector of claim 7.

9. Process for preparing the recombinant proteins of claims 1-5, which comprises the following steps:

a) construction of DNA encoding the desired protein;

b) insertion of DNA in an expression vector;

c) transformation of a host cell with recombinant DNA (rDNA);

d) culture of the transformed host cell so as to express the recombinant protein;

e) extraction and purification of the produced recombinant protein.

10. Process according to claim 9, wherein the host cell is kidney epithelial BOSC cell or SF9 insect cell.

11. Recombinant proteins of claims 1-5 for use as therapeutical agents.

12. Use of recombinant proteins of claims 1-5 in the manufacture of a medicament for the prevention or treatment of chemotherapeutic-induced toxicity.

13. Use according to claim 10, wherein the chemotherapeutic-induced toxicity is myelotoxicity, kidney toxicity, neurotoxicity, mucotoxicity and hepatotoxicity.

14. Pharmaceutical compositions containing an effective amount of the recombinant proteins of claims 1-5, in combination with pharmacologically acceptable excipients.

FIG 1a

```
    1 ATGTGGGTGACCAAACTCCTGCCAGCCCTGCTGCTGCAGCATGTCCTCCTGCATCTCCTC    60
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TACACCCACTGGTTTGAGGACGGTCGGGACGACGACGTCGTACAGGAGGACGTAGAGGAG

    1 M  W  V  T  K  L  L  P  A  L  L  L  Q  H  V  L  L  H  L  L    20

   61 CTGCTCCCCATCGCCATCCCCTATGCAGAGGGACAAAGGAAAAGAAGAAATACAATTCAT   120
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      GACGAGGGGTAGCGGTAGGGGATACGTCTCCCTGTTTCCTTTTCTTCTTTATGTTAAGTA

   21 L  L  P  I  A  I  P  Y  A  E  G  Q  R  K  R  R  N  T  I  H    40

  121 GAATTCAAAAAATCAGCAAAGACTACCCTAATCAAAATAGATCCAGCACTGAAGATAAAA   180
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      CTTAAGTTTTTTAGTCGTTTCTGATGGGATTAGTTTTATCTAGGTCGTGACTTCTATTTT

   41 E  F  K  K  S  A  K  T  T  L  I  K  I  D  P  A  L  K  I  K    60

  181 ACCAAAAAAGTGAATACTGCAGACCAATGTGCTAATAGATGTACTAGGAATAAAGGACTT   240
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TGGTTTTTTCACTTATGACGTCTGGTTACACGATTATCTACATGATCCTTATTTCCTGAA

   61 T  K  K  V  N  T  A  D  Q  C  A  N  R  C  T  R  N  K  G  L    80

  241 CCATTCACTTGCAAGGCTTTTGTTTTTGATAAAGCAAGAAAACAATGCCTCTGGTTCCCC   300
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      GGTAAGTGAACGTTCCGAAAACAAAAACTATTTCGTTCTTTTGTTACGGAGACCAAGGGG

   81 P  F  T  C  K  A  F  V  F  D  K  A  R  K  Q  C  L  W  F  P   100

  301 TTCAATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCATGAATTTGACCTCTATGAA   360
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      AAGTTATCGTACAGTTCACCTCACTTTTTTCTTAAACCGGTACTTAAACTGGAGATACTT

  101 F  N  S  M  S  S  G  V  K  K  E  F  G  H  E  F  D  L  Y  E   120

  361 AACAAAGACTACATTAGAAACTGCATCATTGGTAAAGGACGCAGCTACAAGGGAACAGTA   420
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      TTGTTTCTGATGTAATCTTTGACGTAGTAACCATTTCCTGCGTCGATGTTCCCTTGTCAT

  121 N  K  D  Y  I  R  N  C  I  I  G  K  G  R  S  Y  K  G  T  V   140

  421 TCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGGAGTTCCATGATACCACACGAACAC   480
      ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
      AGATAGTGATTCTCACCGTAGTTTACAGTCGGGACCTCAAGGTACTATGGTGTGCTTGTG

  141 S  I  T  K  S  G  I  K  C  Q  P  W  S  S  M  I  P  H  E  H   160
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(continued)

(continued)


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481  AGCTATCGGGGTAAAGACCTACAGGAAAACTACTGTCGAAATCCTCGAGGGGAAGAAGGG
     --------+---------+---------+---------+---------+---------+
     TCGATAGCCCCATTTCTGGATGTCCTTTTGATGACAGCTTTAGGAGCTCCCCTTCTTCCC  540
161  S  Y  R  G  K  D  L  Q  E  N  Y  C  R  N  P  R  G  E  E  G   180

541  GGACCCTGGTGTTTCACAAGCAATCCAGAGGTACGCTACGAAGTCTGTGACATTCCTCAG
     --------+---------+---------+---------+---------+---------+
     CCTGGGACCACAAAGTGTTCGTTAGGTCTCCATGCGATGCTTCAGACACTGTAAGGAGTC  600
181  G  P  W  C  F  T  S  N  P  E  V  R  Y  E  V  C  D  I  P  Q   200

601  TGTTCAGAAGTTGAATGCATGACCTGCAATGGGGAGAGTTATCGAGGTCTCATGGATCAT
     --------+---------+---------+---------+---------+---------+
     ACAAGTCTTCAACTTACGTACTGGACGTTACCCCTCTCAATAGCTCCAGAGTACCTAGTA  660
201  C  S  E  V  E  C  M  T  C  N  G  E  S  Y  R  G  L  M  D  H   220

661  ACAGAATCAGGCAAGATTTGTCAGCGCTGGGATCATCAGACACCACACCGGCACAAATTC
     --------+---------+---------+---------+---------+---------+
     TGTCTTAGTCCGTTCTAAACAGTCGCGACCCTAGTAGTCTGTGGTGTGGCCGTGTTTAAG  720
221  T  E  S  G  K  I  C  Q  R  W  D  H  Q  T  P  H  R  H  K  F   240

721  TTGCCTGAAAGATATCCCGACAAGGGCTTTGATGATAATTATTGCCGCAATCCCGATGGC
     --------+---------+---------+---------+---------+---------+
     AACGGACTTTCTATAGGGCTGTTCCCGAAACTACTATTAATAACGGCGTTAGGGCTACCG  780
241  L  P  E  R  Y  P  D  K  G  F  D  D  N  Y  C  R  N  P  D  G   260

781  CAGCCGAGGCCATGGTGCTATACTCTTGACCCTCACACCCGCTGGGAGTACTGTGCAATT
     --------+---------+---------+---------+---------+---------+
     GTCGGCTCCGGTACCACGATATGAGAACTGGGAGTGTGGGCGACCCTCATGACACGTTAA  840
261  Q  P  R  P  W  C  Y  T  L  D  P  H  T  R  W  E  Y  C  A  I   280

841  AAAACATGCGCTGACAATACTATGAATGACACTGATGTTCCTTTGGAAACAACTGAATGC
     --------+---------+---------+---------+---------+---------+
     TTTTGTACGCGACTGTTATGATACTTACTGTGACTACAAGGAAACCTTTGTTGACTTACG  900
281  K  T  C  A  D  N  T  M  N  D  T  D  V  P  L  E  T  T  E  C   300

901  ATCCAAGGTCAAGGAGAAGGCTACAGGGGCACTGTCAATACCATTTGGAATGGAATTCCA
     --------+---------+---------+---------+---------+---------+
     TAGGTTCCAGTTCCTCTTCCGATGTCCCCGTGACAGTTATGGTAAACCTTACCTTAAGGT  960
301  I  Q  G  Q  G  E  G  Y  R  G  T  V  N  T  I  W  N  G  I  P   320

961  TGTCAGCGTTGGGATTCTCAGTATCCTCACGAGCATGACATGACTCCTGAAAATTTCAAG
     --------+---------+---------+---------+---------+---------+
     ACAGTCGCAACCCTAAGAGTCATAGGAGTGCTCGTACTGTACTGAGGACTTTTAAAGTTC 1020
321  C  Q  R  W  D  S  Q  Y  P  H  E  H  D  M  T  P  E  N  F  K   340

1021 TGCAAGGACCTACGAGAAAATTACTGCCGAAATCCAGATGGGTCTGAATCACCCTGGTGT
     --------+---------+---------+---------+---------+---------+
     ACGTTCCTGGATGCTCTTTTAATGACGGCTTTAGGTCTACCCAGACTTAGTGGGACCACA 1080
341  C  K  D  L  R  E  N  Y  C  R  N  P  D  G  S  E  S  P  W  C   360

1081 TTTACCACTGATCCAAACATCCGAGTTGGCTACTGCTCCCAAATTCCAAACTGTGATATG
     --------+---------+---------+---------+---------+---------+
     AAATGGTGACTAGGTTTGTAGGCTCAACCGATGACGAGGGTTTAAGGTTTGACACTATAC 1140
361  F  T  T  D  P  N  I  R  V  G  Y  C  S  Q  I  P  N  C  D  M   380
```

(continued)

(continued)

```
1141 TCACATGGACAAGATTGTTATCGTGGGAATGGCAAAAATTATATGGGCAACTTATCCCAA 1200
     ---------+---------+---------+---------+---------+---------+
     AGTGTACCTGTTCTAACAATAGCACCCTTACCGTTTTTAATATACCCGTTGAATAGGGTT

 381 S  H  G  Q  D  C  Y  R  G  N  G  K  N  Y  M  G  N  L  S  Q   400

1201 ACAAGATCTGGACTAACATGTTCAATGTGGGACAAGAACATGGAAGACTTACATCGTCAT 1260
     ---------+---------+---------+---------+---------+---------+
     TGTTCTAGACCTGATTGTACAAGTTACACCCTGTTCTTGTACCTTCTGAATGTAGCAGTA

 401 T  R  S  G  L  T  C  S  M  W  D  K  N  M  E  D  L  H  R  H   420

1261 ATCTTCTGGGAACCAGATGCAAGTAAGCTGAATGAGAATTACTGCCGAAATCCAGATGAT 1320
     ---------+---------+---------+---------+---------+---------+
     TAGAAGACCCTTGGTCTACGTTCATTCGACTTACTCTTAATGACGGCTTTAGGTCTACTA

 421 I  F  W  E  P  D  A  S  K  L  N  E  N  Y  C  R  N  P  D  D   440

1321 GACGCTCATGGACCCTGGTGCTACACGGGAAATCCACTCATTCCTTGGGATTATTGCCCT 1380
     ---------+---------+---------+---------+---------+---------+
     CTGCGAGTACCTGGGACCACGATGTGCCCTTTAGGTGAGTAAGGAACCCTAATAACGGGA

 441 D  A  H  G  P  W  C  Y  T  G  N  P  L  I  P  W  D  Y  C  P   460

1381 ATTTCTCGTTGTGAAGGTGATACCACACCTACAATAGTCAATTTAGACCATCCCGTAATA 1440
     ---------+---------+---------+---------+---------+---------+
     TAAAGAGCAACACTTCCACTATGGTGTGGATGTTATCAGTTAAATCTGGTAGGGCATTAT

 461 I  S  R  C  E  G  D  T  T  P  T  I  V  N  L  D  H  P  V  I   480

1441 TCTTGTGCCAAAACGAAACAATTGCGAGTTGTAAATGGGATTCCAACACGAACAAACATA 1500
     ---------+---------+---------+---------+---------+---------+
     AGAACACGGTTTTGCTTTGTTAACGCTCAACATTTACCCTAAGGTTGTGCTTGTTTGTAT

 481 S  C  A  K  T  K  Q  L  R  V  V  N  G  I  P  T  R  T  N  I   500

1501 GGATGGATGGTTAGTTTGAGATACAGAAATAAACATATCTGCGGAGGATCATTGATAAAG 1560
     ---------+---------+---------+---------+---------+---------+
     CCTACCTACCAATCAAACTCTATGTCTTTATTTGTATAGACGCCTCCTAGTAACTATTTC

 501 G  W  M  V  S  L  R  Y  R  N  K  H  I  C  G  G  S  L  I  K   520

1561 GAGAGTTGGGTTCTTACTGCACGACAGTGTTTCCCTTCTCGAGACTTGAAAGATTATGAA 1620
     ---------+---------+---------+---------+---------+---------+
     CTCTCAACCCAAGAATGACGTGCTGTCACAAAGGGAAGAGCTCTGAACTTTCTAATACTT

 521 E  S  W  V  L  T  A  R  Q  C  F  P  S  R  D  L  K  D  Y  E   540

1621 GCTTGGCTTGGAATTCATGATGTCCACGGAAGAGGAGATGAGAAATGCAAACAGGTTCTC 1680
     ---------+---------+---------+---------+---------+---------+
     CGAACCGAACCTTAAGTACTACAGGTGCCTTCTCCTCTACTCTTTACGTTTGTCCAAGAG

 541 A  W  L  G  I  H  D  V  H  G  R  G  D  E  K  C  K  Q  V  L   560

1681 AATGTTTCCCAGCTGGTATATGGCCCTGAAGGATCAGATCTGGTTTTAATGAAGCTTGCC 1740
     ---------+---------+---------+---------+---------+---------+
     TTACAAAGGGTCGACCATATACCGGGACTTCCTAGTCTAGACCAAAATTACTTCGAACGG

 561 N  V  S  Q  L  V  Y  G  P  E  G  S  D  L  V  L  M  K  L  A   580

1741 AGGCCTGCTGTCCTGGATGATTTTGTTAGTACGATTGATTTACCTAATTATGGATGCACA 1800
     ---------+---------+---------+---------+---------+---------+
     TCCGGACGACAGGACCTACTAAAACAATCATGCTAACTAAATGGATTAATACCTACGTGT

 581 R  P  A  V  L  D  D  F  V  S  T  I  D  L  P  N  Y  G  C  T   600
```

(continued)

(continued)

```
1801  ATTCCTGAAAAGACCAGTTGCAGTGTTTATGGCTGGGGCTACACTGGATTGATCAACTAT  1860
      ---------+---------+---------+---------+---------+---------+
      TAAGGACTTTTCTGGTCAACGTCACAAATACCGACCCCGATGTGACCTAACTAGTTGATA

 601  I  P  E  K  T  S  C  S  V  Y  G  W  G  Y  T  G  L  I  N  Y   620

1861  GATGGCCTATTACGAGTGGCACATCTCTATATAATGGGAAATGAGAAATGCAGCCAGCAT  1920
      ---------+---------+---------+---------+---------+---------+
      CTACCGGATAATGCTCACCGTGTAGAGATATATTACCCTTTACTCTTTACGTCGGTCGTA

 621  D  G  L  L  R  V  A  H  L  Y  I  M  G  N  E  K  C  S  Q  H   640

1921  CATCGAGGGAAGGTGACTCTGAATGAGTCTGAAATATGTGCTGGGGCTGAAAAGATTGGA  1980
      ---------+---------+---------+---------+---------+---------+
      GTAGCTCCCTTCCACTGAGACTTACTCAGACTTTATACACGACCCCGACTTTTCTAACCT

 641  H  R  G  K  V  T  L  N  E  S  E  I  C  A  G  A  E  K  I  G   660

1981  TCAGGACCATGTGAGGGGGATTATGGTGGCCCACTTGTTTGTGAGCAACATAAAATGAGA  2040
      ---------+---------+---------+---------+---------+---------+
      AGTCCTGGTACACTCCCCCTAATACCACCGGGTGAACAAACACTCGTTGTATTTTACTCT

 661  S  G  P  C  E  G  D  Y  G  G  P  L  V  C  E  Q  H  K  M  R   680

2041  ATGGTTCTTGGTGTCATTGTTCCTGGTCGTGGATGTGCCATTCCAAATCGTCCTGGTATT  2100
      ---------+---------+---------+---------+---------+---------+
      TACCAAGAACCACAGTAACAAGGACCAGCACCTACACGGTAAGGTTTAGCAGGACCATAA

 681  M  V  L  G  V  I  V  P  G  R  G  C  A  I  P  N  R  P  G  I   700

2101  TTTGTCCGAGTAGCATATTATGCAAAATGGATACACAAAATTATTTTAACATATAAGGTA  2160
      ---------+---------+---------+---------+---------+---------+
      AAACAGGCTCATCGTATAATACGTTTTACCTATGTGTTTTAATAAAATTGTATATTCCAT

 701  F  V  R  V  A  Y  Y  A  K  W  I  H  K  I  I  L  T  Y  K  V   720

2161  CCACAGTCATAG  2172
      ---------+==
      GGTGTCAGTATC

 721  P  Q  S  *    723
```

FIG 1b

```
    1 ATGGGGTGGCTCCCACTCCTGCTGCTTCTGACTCAATGCTTAGGGGTCCCTGGGCAGCGC   60
      ---------+---------+---------+---------+---------+---------+
      TACCCCACCGAGGGTGAGGACGACGAAGACTGAGTTACGAATCCCCAGGGACCCGTCGCG

    1 M  G  W  L  P  L  L  L  L  L  T  Q  C  L  G  V  P  G  Q  R    20

   61 TCGCCATTGAATGACTTCCAAGTGCTCCGGGGCACAGAGCTACAGCACCTGCTACATGCG  120
      ---------+---------+---------+---------+---------+---------+
      AGCGGTAACTTACTGAAGGTTCACGAGGCCCCGTGTCTCGATGTCGTGGACGATGTACGC

   21 S  P  L  N  D  F  Q  V  L  R  G  T  E  L  Q  H  L  L  H  A    40

  121 GTGGTGCCCGGGCCTTGGCAGGAGGATGTGGCAGATGCTGAAGAGTGTGCTGGTCGCTGT  180
      ---------+---------+---------+---------+---------+---------+
      CACCACGGGCCCGGAACCGTCCTCCTACACCGTCTACGACTTCTCACACGACCAGCGACA

   41 V  V  P  G  P  W  Q  E  D  V  A  D  A  E  E  C  A  G  R  C    60

  181 GGGCCCTTAATGGACTGCCGGGCCTTCCACTACAACGTGAGCAGCCATGGTTGCCAACTG  240
      ---------+---------+---------+---------+---------+---------+
      CCCGGGAATTACCTGACGGCCCGGAAGGTGATGTTGCACTCGTCGGTACCAACGGTTGAC

   61 G  P  L  M  D  C  R  A  F  H  Y  N  V  S  S  H  G  C  Q  L    80

  241 CTGCCATGGACTCAACACTCGCCCCACACGAGGCTGCGGCGTTCTGGGCGCTGTGACCTC  300
      ---------+---------+---------+---------+---------+---------+
      GACGGTACCTGAGTTGTGAGCGGGGTGTGCTCCGACGCCGCAAGACCCGCGACACTGGAG

   81 L  P  W  T  Q  H  S  P  H  T  R  L  R  R  S  G  R  C  D  L   100

  301 TTCCAGAAGAAAGACTACGTACGGACCTGCATCATGAACAATGGGGTTGGGTACCGGGGC  360
      ---------+---------+---------+---------+---------+---------+
      AAGGTCTTCTTTCTGATGCATGCCTGGACGTAGTACTTGTTACCCCAACCCATGGCCCCG

  101 F  Q  K  K  D  Y  V  R  T  C  I  M  N  N  G  V  G  Y  R  G   120

  361 ACCATGGCCACGACCGTGGGTGGCCTGCCCTGCCAGGCTTGGAGCCACAAGTTCCCGAAT  420
      ---------+---------+---------+---------+---------+---------+
      TGGTACCGGTGCTGGCACCCACCGGACGGGACGGTCCGAACCTCGGTGTTCAAGGGCTTA

  121 T  M  A  T  T  V  G  G  L  P  C  Q  A  W  S  H  K  F  P  N   140

  421 GATCACAAGTACACGCCCACTCTCCGGAATGGCCTGGAAGAGAACTTCTGCCGTAACCCT  480
      ---------+---------+---------+---------+---------+---------+
      CTAGTGTTCATGTGCGGGTGAGAGGCCTTACCGGACCTTCTCTTGAAGACGGCATTGGGA

  141 D  H  K  Y  T  P  T  L  R  N  G  L  E  E  N  F  C  R  N  P   160
```

(continued)

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```
481  GATGGCGACCCCGGAGGTCCTTGGTGCTACACAACAGACCCTGCTGTGCGCTTCCAGAGC  540
     CTACCGCTGGGGCCTCCAGGAACCACGATGTGTTGTCTGGGACGACACGCGAAGGTCTCG

161  D  G  D  P  G  G  P  W  C  Y  T  T  D  P  A  V  R  F  Q  S   180

531  TGCGGCATCAAATCCTGCCGGGAGGCCGCGTGTGTCTGGTGCAATGGCGAGGAATACCGC  600
     ACGCCGTAGTTTAGGACGGCCCTCCGGCGCACACAGACCACGTTACCGCTCCTTATGGCG

181  C  G  I  K  S  C  R  E  A  A  C  V  W  C  N  G  E  E  Y  R   200

601  GGCGCGGTAGACCGCACGGAGTCAGGGCGCGAGTGCCAGCGCTGGGATCTTCAGCACCCG  660
     CCGCGCCATCTGGCGTGCCTCAGTCCCGCGCTCACGGTCGCGACCCTAGAAGTCGTGGGC

201  G  A  V  D  R  T  E  S  G  R  E  C  Q  R  W  D  L  Q  H  P   220

661  CACCAGCACCCCTTCGAGCCGGGCAAGTTCCTCGACCAAGGTCTGGACGACAACTATTGC  720
     GTGGTCGTGGGGAAGCTCGGCCCGTTCAAGGAGCTGGTTCCAGACCTGCTGTTGATAACG

221  H  Q  H  P  F  E  P  G  K  F  L  D  Q  G  L  D  D  N  Y  C   240

721  CGGAATCCTGACGGCTCCGAGCGGCCATGGTGCTACACTACGGATCCGCAGATCGAGCGA  780
     GCCTTAGGACTGCCGAGGCTCGCCGGTACCACGATGTGATGCCTAGGCGTCTAGCTCGCT

241  R  N  P  D  G  S  E  R  P  W  C  Y  T  T  D  P  Q  I  E  R   260

781  GAGTTCTGTGACCTCCCCCGCTGCGGGTCCGAGGCACAGCCCCGCCAAGAGGCCACAACT  840
     CTCAAGACACTGGAGGGGGCGACGCCCAGGCTCCGTGTCGGGGCGGTTCTCCGGTGTTGA

261  E  F  C  D  L  P  R  C  G  S  E  A  Q  P  R  Q  E  A  T  T   280

841  GTCAGCTGCTTCCGCGGGAAGGGTGAGGGCTACCGGGGCACAGCCAATACCACCACTGCG  900
     CAGTCGACGAAGGCGCCCTTCCCACTCCCGATGGCCCCGTGTCGGTTATGGTGGTGACGC

281  V  S  C  F  R  G  K  G  E  G  Y  R  G  T  A  N  T  T  T  A   300

901  GGCGTACCTTGCCAGCGTTGGGACGCGCAAATCCCGCATCAGCACCGATTTACGCCAGAA  960
     CCGCATGGAACGGTCGCAACCCTGCGCGTTTAGGGCGTAGTCGTGGCTAAATGCGGTCTT

301  G  V  P  C  Q  R  W  D  A  Q  I  P  H  Q  H  R  F  T  P  E   320

961  AAATACGCGTGCAAAGACCTTCGGGAGAACTTCTGCCGGAACCCCGACGGCTCAGAGGCG  1020
     TTTATGCGCACGTTTCTGGAAGCCCTCTTGAAGACGGCCTTGGGGCTGCCGAGTCTCCGC

321  K  Y  A  C  K  D  L  R  E  N  F  C  R  N  P  D  G  S  E  A   340

1021 CCCTGGTGCTTCACACTGCGGCCCGGCATGCGCGCGGCCTTTTGCTACCAGATCCGGCGT  1080
     GGGACCACGAAGTGTGACGCCGGGCCGTACGCGCGCCGGAAAACGATGGTCTAGGCCGCA

341  P  W  C  F  T  L  R  P  G  M  R  A  A  F  C  Y  Q  I  R  R   360

1081 TGTACAGACGACGTGCGGCCCCAGGACTGCTACCACGGCGCAGGGGAGCAGTACCGCGGC  1140
     ACATGTCTGCTGCACGCCGGGGTCCTGACGATGGTGCCGCGTCCCCTCGTCATGGCGCCG

361  C  T  D  D  V  R  P  Q  D  C  Y  H  G  A  G  E  Q  Y  R  G   380
```

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```
1141 ACGGTCAGCAAGACCCGCAAGGGTGTCCAGTGCCAGCGCTGGTCCGCTGAGACGCCGCAC 1200
     TGCCAGTCGTTCTGGGCGTTCCCACAGGTCACGGTCGCGACCAGGCGACTCTGCGGCGTG
 381 T  V  S  K  T  R  K  G  V  Q  C  Q  R  W  S  A  E  T  P  H  400

1201 AAGCCGCAGTTCACGTTTACCTCCGAACCGCATGCACAACTGGAGGAGAACTTCTGCCGG 1260
     TTCGGCGTCAAGTGCAAATGGAGGCTTGGCGTACGTGTTGACCTCCTCTTGAAGACGGCC
 401 K  P  Q  F  T  F  T  S  E  P  H  A  Q  L  E  E  N  F  C  R  420

1261 AACCCAGATGGGGATAGCCATGGGCCCTGGTGCTACACGATGGACCCAAGGACCCCATTC 1320
     TTGGGTCTACCCCTATCGGTACCCGGGACCACGATGTGCTACCTGGGTTCCTGGGGTAAG
 421 N  P  D  G  D  S  H  G  P  W  C  Y  T  M  D  P  R  T  P  F  440

1321 GACTACTGTGCCCTGCGACGCTGCGCTGATGACCAGCCGCCATCAATCCTGGACCCCCCA 1380
     CTGATGACACGGGACGCTGCGACGCGACTACTGGTCGGCGGTAGTTAGGACCTGGGGGGT
 441 D  Y  C  A  L  R  R  C  A  D  D  Q  P  P  S  I  L  D  P  P  460

1381 GACCAGGTGCAGTTTGAGAAGTGTGGCAAGAGGGTGGATCGGCTGGATCAGCGGCGTTCC 1440
     CTGGTCCACGTCAAACTCTTCACACCGTTCTCCCACCTAGCCGACCTAGTCGCCGCAAGG
 461 D  Q  V  Q  F  E  K  C  G  K  R  V  D  R  L  D  Q  R  R  S  480

1441 AAGCTGCGCGTGGTTGGGGGCCATCCGGGCAACTCACCCTGGACAGTCAGCTTGCGGAAT 1500
     TTCGACGCGCACCAACCCCCGGTAGGCCCGTTGAGTGGGACCTGTCAGTCGAACGCCTTA
 481 K  L  R  V  V  G  G  H  P  G  N  S  P  W  T  V  S  L  R  N  500

1501 CGGCAGGGCCAGCATTTCTGCGGGGGGTCTCTAGTGAAGGAGCAGTGGATACTGACTGCC 1560
     GCCGTCCCGGTCGTAAAGACGCCCCCCAGAGATCACTTCCTCGTCACCTATGACTGACGG
 501 R  Q  G  Q  H  F  C  G  G  S  L  V  K  E  Q  W  I  L  T  A  520

1561 CGGCAGTGCTTCTCCTCCTGCCATATGCCTCTCACGGGCTATGAGGTATGGTTGGGCACC 1620
     GCCGTCACGAAGAGGAGGACGGTATACGGAGAGTGCCCGATACTCCATACCAACCCGTGG
 521 R  Q  C  F  S  S  C  H  M  P  L  T  G  Y  E  V  W  L  G  T  540

1621 CTGTTCCAGAACCCACAGCATGGAGAGCCAAGCCTACAGCGGGTCCCAGTAGCCAAGATG 1680
     GACAAGGTCTTGGGTGTCGTACCTCTCGGTTCGGATGTCGCCCAGGGTCATCGGTTCTAC
 541 L  F  Q  N  P  Q  H  G  E  P  S  L  Q  R  V  P  V  A  K  M  560

1681 GTGTGTGGGCCCTCAGGCTCCCAGCTTGTCCTGCTCAAGCTGGAGAGATCTGTGACCCTG 1740
     CACACACCCGGGAGTCCGAGGGTCGAACAGGACGAGTTCGACCTCTCTAGACACTGGGAC
 561 V  C  G  P  S  G  S  Q  L  V  L  L  K  L  E  R  S  V  T  L  580

1741 AACCAGCGTGTGGCCCTGATCTGCCTGCCCCCTGAATGGTATGTGGTGCCTCCAGGGACC 1800
     TTGGTCGCACACCGGGACTAGACGGACGGGGGACTTACCATACACCACGGAGGTCCCTGG
 581 N  Q  R  V  A  L  I  C  L  P  P  E  W  Y  V  V  P  P  G  T  600
```

(continued)

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```
                AAGTGTGAGATTGCAGGCTGGGGTGAGACCAAAGGTACGGGTAATGACACAGTCCTAAAT
1801            ---------+---------+---------+---------+---------+---------+ 1860
                TTCACACTCTAACGTCCGACCCCACTCTGGTTTCCATGCCCATTACTGTGTCAGGATTTA
 601  K  C  E  I  A  G  W  G  E  T  K  G  T  G  N  D  T  V  L  N    620

                GTGGCCTTTCTGAATGTTATCTCCAACCAGGAGTGTAACATCAAGCACCGAGGACGTGTG
1861            ---------+---------+---------+---------+---------+---------+ 1920
                CACCGGAAAGACTTACAATAGAGGTTGGTCCTCACATTGTAGTTCGTGGCTCCTGCACAC
 621  V  A  F  L  N  V  I  S  N  Q  E  C  N  I  K  H  R  G  R  V    640

                CGGGAGAGTGAGATGTGCACTGAGGGACTGTTGGCCCCTGTGGGGGCCTGTGAGGGTGAC
1921            ---------+---------+---------+---------+---------+---------+ 1980
                GCCCTCTCACTCTACACGTGACTCCCTGACAACCGGGGACACCCCCGGACACTCCCACTG
 641  R  E  S  E  M  C  T  E  G  L  L  A  P  V  G  A  C  E  G  D    660

                TACGGGGGCCCACTTGCCTGCTTTACCCACAACTGCTGGGTCCTGGAAGGAATTATAATC
1981            ---------+---------+---------+---------+---------+---------+ 2040
                ATGCCCCCGGGTGAACGGACGAAATGGGTGTTGACGACCCAGGACCTTCCTTAATATTAG
 661  Y  G  G  P  L  A  C  F  T  H  N  C  W  V  L  E  G  I  I  I    680

                CCCAACCGAGTATGCGCAAGGTCCCGCTGGCCAGCTGTCTTCACGCGTGTCTCTGTGTTT
2041            ---------+---------+---------+---------+---------+---------+ 2100
                GGGTTGGCTCATACGCGTTCCAGGGCGACCGGTCGACAGAAGTGCGCACAGAGACACAAA
 681  P  N  R  V  C  A  R  S  R  W  P  A  V  F  T  R  V  S  V  F    700

                GTGGACTGGATTCACAAGGTCATGAGACTGGGTTAG
2101            ---------+---------+---------+---=== 2136
                CACCTGACCTAAGTGTTCCAGTACTCTGACCCAATC
 701  V  D  W  I  H  K  V  M  R  L  G  *    711
```

FIG 2a

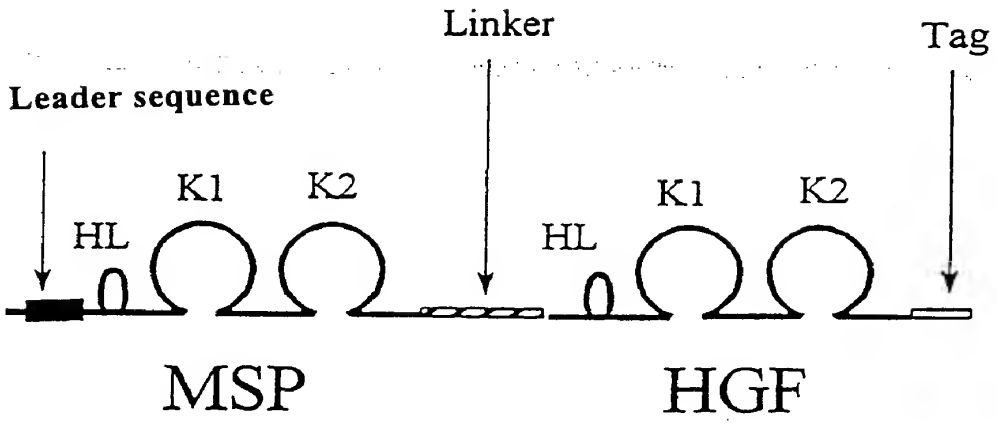

FIG 2b

```
    1 GAATTCCACCATGGGGTGGCTCCCACTCCTGCTGCTTCTGACTCAATGCTTAGGGGTCCC   60
      ---------+---------+---------+---------+---------+---------+---------+
      CTTAAGGTGGTACCCCACCGAGGGTGAGGACGACGAAGACTGAGTTACGAATCCCCAGGG

    1           M  G  W  L  P  L  L  L  L  L  T  Q  C  L  G  V  P   17

   61 TGGGCAGCGCTCGCCATTGAATGACTTCCAAGTGCTCCGGGGCACAGAGCTACAGCACCT  120
      ---------+---------+---------+---------+---------+---------+---------+
      ACCCGTCGCGAGCGGTAACTTACTGAAGGTTCACGAGGCCCCGTGTCTCGATGTCGTGGA

   18  G  Q  R  S  P  L  N  D  F  Q  V  L  R  G  T  E  L  Q  H  L   37

  121 GCTACATGCGGTGGTGCCCGGGCCTTGGCAGGAGGATGTGGCAGATGCTGAAGAGTGTGC  180
      ---------+---------+---------+---------+---------+---------+---------+
      CGATGTACGCCACCACGGGCCCGGAACCGTCCTCCTACACCGTCTACGACTTCTCACACG

   38  L  H  A  V  V  P  G  P  W  Q  E  D  V  A  D  A  E  E  C  A   57

  181 TGGTCGCTGTGGGCCCTTAATGGACTGCCGGGCCTTCCACTACAACGTGAGCAGCCATGG  240
      ---------+---------+---------+---------+---------+---------+---------+
      ACCAGCGACACCCGGGAATTACCTGACGGCCCGGAAGGTGATGTTGCACTCGTCGGTACC

   58  G  R  C  G  P  L  M  D  C  R  A  F  H  Y  N  V  S  S  H  G   77

  241 TTGCCAACTGCTGCCATGGACTCAACACTCGCCCCACACGAGGCTGCGGCGTTCTGGGCG  300
      ---------+---------+---------+---------+---------+---------+---------+
      AACGGTTGACGACGGTACCTGAGTTGTGAGCGGGGTGTGCTCCGACGCCGCAAGACCCGC

   78  C  Q  L  L  P  W  T  Q  H  S  P  H  T  R  L  R  R  S  G  R   97

  301 CTGTGACCTCTTCCAGAAGAAAGACTACGTACGGACCTGCATCATGAACAATGGGGTTGG  360
      ---------+---------+---------+---------+---------+---------+---------+
      GACACTGGAGAAGGTCTTCTTTCTGATGCATGCCTGGACGTAGTACTTGTTACCCCAACC

   98  C  D  L  F  Q  K  K  D  Y  V  R  T  C  I  M  N  N  G  V  G  117

  361 GTACCGGGGCACCATGGCCACGACCGTGGGTGGCCTGCCCTGCCAGGCTTGGAGCCACAA  420
      ---------+---------+---------+---------+---------+---------+---------+
      CATGGCCCCGTGGTACCGGTGCTGGCACCCACCGGACGGGACGGTCCGAACCTCGGTGTT

  118  Y  R  G  T  M  A  T  T  V  G  G  L  P  C  Q  A  W  S  H  K  137

  421 GTTCCCGAATGATCACAAGTACACGCCCACTCTCCGGAATGGCCTGGAAGAGAACTTCTG  480
      ---------+---------+---------+---------+---------+---------+---------+
      CAAGGGCTTACTAGTGTTCATGTGCGGGTGAGAGGCCTTACCGGACCTTCTCTTGAAGAC

  138  F  P  N  D  H  K  Y  T  P  T  L  R  N  G  L  E  E  N  F  C  157

  481 CCGTAACCCTGATGGCGACCCCGGAGGTCCTTGGTGCTACACAACAGACCCTGCTGTGCG  540
      ---------+---------+---------+---------+---------+---------+---------+
      GGCATTGGGACTACCGCTGGGGCCTCCAGGAACCACGATGTGTTGTCTGGGACGACACGC

  158  R  N  P  D  G  D  P  G  G  P  W  C  Y  T  T  D  P  A  V  R  177
```

(continued)

(continued)

```
541  CTTCCAGAGCTGCGGCATCAAATCCTGCCGGGAGGCCGCGTGTGTCTGGTGCAATGGCGA
     GAAGGTCTCGACGCCGTAGTTTAGGACGGCCCTCCGGCGCACACAGACCACGTTACCGCT
178   F  Q  S  C  G  I  K  S  C  R  E  A  A  C  V  W  C  N  G  E   197

601  GGAATACCGCGGCGCGGTAGACCGCACGGAGTCAGGGCGCGAGTGCCAGCGCTGGGATCT
     CCTTATGGCGCCGCGCCATCTGGCGTGCCTCAGTCCCGCGCTCACGGTCGCGACCCTAGA
198   E  Y  R  G  A  V  D  R  T  E  S  G  R  E  C  Q  R  W  D  L   217

661  TCAGCACCCGCACCAGCACCCCTTCGAGCCGGGCAAGTTCCTCGACCAAGGTCTGGACGA
     AGTCGTGGGCGTGGTCGTGGGGAAGCTCGGCCCGTTCAAGGAGCTGGTTCCAGACCTGCT
218   Q  H  P  H  Q  H  P  F  E  P  G  K  F  L  D  Q  G  L  D  D   237

721  CAACTATTGCCGGAATCCTGACGGCTCCGAGCGGCCATGGTGCTACACTACGGATCCGCA
     GTTGATAACGGCCTTAGGACTGCCGAGGCTCGCCGGTACCACGATGTGATGCCTAGGCGT
238   N  Y  C  R  N  P  D  G  S  E  R  P  W  C  Y  T  T  D  P  Q   257

781  GATCGAGCGAGAGTTCTGTGACCTCCCCCGCTGCGGGTCCGAGGCACAGCCCCGCCTCGA
     CTAGCTCGCTCTCAAGACACTGGAGGGGGCGACGCCCAGGCTCCGTGTCGGGGCGGAGCT
258   I  E  R  E  F  C  D  L  P  R  C  G  S  E  A  Q  P  R  L  E   277

841  GGGCGGTGGCGGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCTCTAGAGGGACAAAG
     CCCGCCACCGCCAAGACCACCGCCACCGAGGCCGCCACCGCCAAGAGATCTCCCTGTTTC
278   G  G  G  G  S  G  G  G  G  S  G  G  G  G  S  L  E  G  Q  R   297

901  GAAAAGAAGAAATACAATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAATCAAAAT
     CTTTTCTTCTTTATGTTAAGTACTTAAGTTTTTTAGTCGTTTCTGATGGGATTAGTTTTA
298   K  R  R  N  T  I  H  E  F  K  K  S  A  K  T  T  L  I  K  I   317

961  AGATCCAGCACTGAAGATAAAAACCAAAAAAGTGAATACTGCAGACCAATGTGCTAATAG
     TCTAGGTCGTGACTTCTATTTTTGGTTTTTTCACTTATGACGTCTGGTTACACGATTATC
318   D  P  A  L  K  I  K  T  K  K  V  N  T  A  D  Q  C  A  N  R   337

1021 ATGTACTAGGAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTTTGATAAAGCAAG
     TACATGATCCTTATTTCCTGAAGGTAAGTGAACGTTCCGAAAACAAAAACTATTTCGTTC
338   C  T  R  N  K  G  L  P  F  T  C  K  A  F  V  F  D  K  A  R   357

1081 AAAACAATGCCTCTGGTTCCCCTTCAATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGG
     TTTTGTTACGGAGACCAAGGGGAAGTTATCGTACAGTTCACCTCACTTTTTTCTTAAACC
358   K  Q  C  L  W  F  P  F  N  S  M  S  S  G  V  K  K  E  F  G   377

1141 CCATGAATTTGACCTCTATGAAAACAAAGACTACATTAGAAACTGCATCATTGGTAAAGG
     GGTACTTAAACTGGAGATACTTTTGTTTCTGATGTAATCTTTGACGTAGTAACCATTTCC
378   H  E  F  D  L  Y  E  N  K  D  Y  I  R  N  C  I  I  G  K  G   397
```

(continued)

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```
1201  ACGCAGCTACAAGGGAACAGTATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGGAG  1260
      TGCGTCGATGTTCCCTTGTCATAGATAGTGATTCTCACCGTAGTTTACAGTCGGGACCTC

 398  R  S  Y  K  G  T  V  S  I  T  K  S  G  I  K  C  Q  P  W  S   417

1261  TTCCATGATACCACACGAACACAGCTATCGGGGTAAAGACCTACAGGAAAACTACTGTCG  1320
      AAGGTACTATGGTGTGCTTGTGTCGATAGCCCCATTTCTGGATGTCCTTTTGATGACAGC

 418  S  M  I  P  H  E  H  S  Y  R  G  K  D  L  Q  E  N  Y  C  R   437

1321  AAATCCTCGAGGGGAAGAAGGGGGACCCTGGTGTTTCACAAGCAATCCAGAGGTACGCTA  1380
      TTTAGGAGCTCCCCTTCTTCCCCCTGGGACCACAAAGTGTTCGTTAGGTCTCCATGCGAT

 438  N  P  R  G  E  E  G  G  P  W  C  F  T  S  N  P  E  V  R  Y   457

1381  CGAAGTCTGTGACATTCCTCAGTGTTCAGAAGTTGAATGCATGACCTGCAATGGGGAGAG  1440
      GCTTCAGACACTGTAAGGAGTCACAAGTCTTCAACTTACGTACTGGACGTTACCCCTCTC

 458  E  V  C  D  I  P  Q  C  S  E  V  E  C  M  T  C  N  G  E  S   477

1441  TTATCGAGGTCTCATGGATCATACAGAATCAGGCAAGATTTGTCAGCGCTGGGATCATCA  1500
      AATAGCTCCAGAGTACCTAGTATGTCTTAGTCCGTTCTAAACAGTCGCGACCCTAGTAGT

 478  Y  R  G  L  M  D  H  T  E  S  G  K  I  C  Q  R  W  D  H  Q   497

1501  GACACCACACCGGCACAAATTCTTGCCTGAAAGATATCCCGACAAGGGCTTTGATGATAA  1560
      CTGTGGTGTGGCCGTGTTTAAGAACGGACTTTCTATAGGGCTGTTCCCGAAACTACTATT

 498  T  P  H  R  H  K  F  L  P  E  R  Y  P  D  K  G  F  D  D  N   517

1561  TTATTGCCGCAATCCCGATGGCCAGCCGAGGCCATGGTGCTATACTCTTGACCCTCACAC  1620
      AATAACGGCGTTAGGGCTACCGGTCGGCTCCGGTACCACGATATGAGAACTGGGAGTGTG

 518  Y  C  R  N  P  D  G  Q  P  R  P  W  C  Y  T  L  D  P  H  T   537

1621  CCGCTGGGAGTACTGTGCAATTAAAACATGCGCTGACAAAGCTGACGACGACGACAAACA  1680
      GGCGACCCTCATGACACGTTAATTTTGTACGCGACTGTTTCGACTGCTGCTGCTGTTTGT

 538  R  W  E  Y  C  A  I  K  T  C  A  D  K  A  D  D  D  D  K  H   557

1681  CCACCACCACCACCACCACTAGGGTCGAC  1709
      GGTGGTGGTGGTGGTGGTGATCCCAGCTG

 558  H  H  H  H  H  H  *            563
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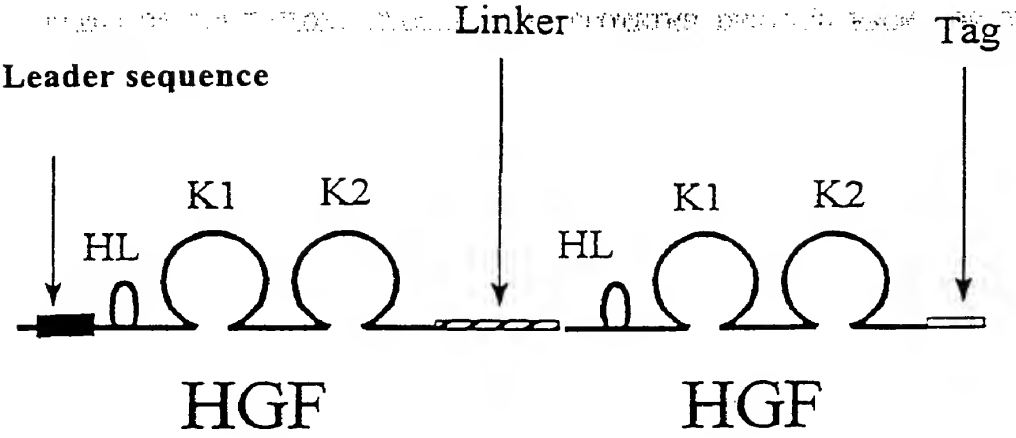
FIG 3a



14/19

FIG 3b

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1   GGATCCGCCAGCCCGTCCAGCAGCACCATGTGGGTGACCAAACTCCTGCCAGCCCTGCTG   60
    ---------+---------+---------+---------+---------+---------+
    CCTAGGCGGTCGGGCAGGTCGTCGTGGTACACCCACTGGTTTGAGGACGGTCGGGACGAC

1                                   M  W  V  T  K  L  L  P  A  L  L   11

61  CTGCAGCATGTCCTCCTGCATCTCCTCCTGCTCCCCATCGCCATCCCCTATGCAGAGGGA   120
    ---------+---------+---------+---------+---------+---------+
    GACGTCGTACAGGAGGACGTAGAGGAGGACGAGGGGTAGCGGTAGGGGATACGTCTCCCT

12  L  Q  H  V  L  L  H  L  L  L  L  P  I  A  I  P  Y  A  E  G   31

121 CAAAGGAAAAGAAGAAATACAATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAATC   180
    ---------+---------+---------+---------+---------+---------+
    GTTTCCTTTTCTTCTTTATGTTAAGTACTTAAGTTTTTTAGTCGTTTCTGATGGGATTAG

32  Q  R  K  R  R  N  T  I  H  E  F  K  K  S  A  K  T  T  L  I   51

181 AAAATAGATCCAGCACTGAAGATAAAAACCAAAAAAGTGAATACTGCAGACCAATGTGCT   240
    ---------+---------+---------+---------+---------+---------+
    TTTTATCTAGGTCGTGACTTCTATTTTTGGTTTTTTCACTTATGACGTCTGGTTACACGA

52  K  I  D  P  A  L  K  I  K  T  K  K  V  N  T  A  D  Q  C  A   71

241 AATAGATGTACTAGGAATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTTTGATAAA   300
    ---------+---------+---------+---------+---------+---------+
    TTATCTACATGATCCTTATTTCCTGAAGGTAAGTGAACGTTCCGAAAACAAAAACTATTT

72  N  R  C  T  R  N  K  G  L  P  F  T  C  K  A  F  V  F  D  K   91

301 GCAAGAAAACAATGCCTCTGGTTCCCCTTCAATAGCATGTCAAGTGGAGTGAAAAAAGAA   360
    ---------+---------+---------+---------+---------+---------+
    CGTTCTTTTGTTACGGAGACCAAGGGGAAGTTATCGTACAGTTCACCTCACTTTTTTCTT

92  A  R  K  Q  C  L  W  F  P  F  N  S  M  S  S  G  V  K  K  E   111

361 TTTGGCCATGAATTTGACCTCTATGAAAACAAAGACTACATTAGAAACTGCATCATTGGT   420
    ---------+---------+---------+---------+---------+---------+
    AAACCGGTACTTAAACTGGAGATACTTTTGTTTCTGATGTAATCTTTGACGTAGTAACCA

112 F  G  H  E  F  D  L  Y  E  N  K  D  Y  I  R  N  C  I  I  G   131

421 AAAGGACGCAGCTACAAGGGAACAGTATCTATCACTAAGAGTGGCATCAAATGTCAGCCC   480
    ---------+---------+---------+---------+---------+---------+
    TTTCCTGCGTCGATGTTCCCTTGTCATAGATAGTGATTCTCACCGTAGTTTACAGTCGGG

132 K  G  R  S  Y  K  G  T  V  S  I  T  K  S  G  I  K  C  Q  P   151

481 TGGAGTTCCATGATACCACACGAACACAGCTATCGGGGTAAAGACCTACAGGAAAACTAC   540
    ---------+---------+---------+---------+---------+---------+
    ACCTCAAGGTACTATGGTGTGCTTGTGTCGATAGCCCCATTTCTGGATGTCCTTTTGATG

152 W  S  S  M  I  P  H  E  H  S  Y  R  G  K  D  L  Q  E  N  Y   171
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(continued)

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541  TGTCGAAATCCTCGAGGGGAAGAAGGGGGACCCTGGTGTTTCACAAGCAATCCAGAGGTA
     ACAGCTTTAGGAGCTCCCCTTCTTCCCCCTGGGACCACAAAGTGTTCGTTAGGTCTCCAT  600
172  C  R  N  P  R  G  E  E  G  G  P  W  C  F  T  S  N  P  E  V   191

601  CGCTACGAAGTCTGTGACATTCCTCAGTGTTCAGAAGTTGAATGCATGACCTGCAATGGG
     GCGATGCTTCAGACACTGTAAGGAGTCACAAGTCTTCAACTTACGTACTGGACGTTACCC  660
192  R  Y  E  V  C  D  I  P  Q  C  S  E  V  E  C  M  T  C  N  G   211

661  GAGAGTTATCGAGGTCTCATGGATCATACAGAATCAGGCAAGATTTGTCAGCGCTGGGAT
     CTCTCAATAGCTCCAGAGTACCTAGTATGTCTTAGTCCGTTCTAAACAGTCGCGACCCTA  720
212  E  S  Y  R  G  L  M  D  H  T  E  S  G  K  I  C  Q  R  W  D   231

721  CATCAGACACCACACCGGCACAAATTCTTGCCTGAAAGATATCCCGACAAGGGCTTTGAT
     GTAGTCTGTGGTGTGGCCGTGTTTAAGAACGGACTTTCTATAGGGCTGTTCCCGAAACTA  780
232  H  Q  T  P  H  R  H  K  F  L  P  E  R  Y  P  D  K  G  F  D   251

781  GATAATTATTGCCGCAATCCCGATGGCCAGCCGAGGCCATGGTGCTATACTCTTGACCCT
     CTATTAATAACGGCGTTAGGGCTACCGGTCGGCTCCGGTACCACGATATGAGAACTGGGA  840
252  D  N  Y  C  R  N  P  D  G  Q  P  R  P  W  C  Y  T  L  D  P   271

841  CACACCCGCTGGGAGTACTGTGCAATTAAAACATGCGCTGACAAAGCTTCGGGCGGTGGC
     GTGTGGGCGACCCTCATGACACGTTAATTTTGTACGCGACTGTTTCGAAGCCCGCCACCG  900
272  H  T  R  W  E  Y  C  A  I  K  T  C  A  D  K  A  S  G  G  G   291

901  GGTTCTGGTGGCGGTGGCTCCGGCGGTGGCGGTTCTCTAGAGGGACAAAGGAAAAGAAGA
     CCAAGACCACCGCCACCGAGGCCGCCACCGCCAAGAGATCTCCCTGTTTCCTTTTCTTCT  960
292  G  S  G  G  G  G  S  G  G  G  G  S  L  E  G  Q  R  K  R  R   311

961  AATACAATTCATGAATTCAAAAAATCAGCAAAGACTACCCTAATCAAAATAGATCCAGCA
     TTATGTTAAGTACTTAAGTTTTTTAGTCGTTTCTGATGGGATTAGTTTTATCTAGGTCGT  1020
312  N  T  I  H  E  F  K  K  S  A  K  T  T  L  I  K  I  D  P  A   331

1021 CTGAAGATAAAAACCAAAAAAGTGAATACTGCAGACCAATGTGCTAATAGATGTACTAGG
     GACTTCTATTTTTGGTTTTTTCACTTATGACGTCTGGTTACACGATTATCTACATGATCC  1080
332  L  K  I  K  T  K  K  V  N  T  A  D  Q  C  A  N  R  C  T  R   351

1081 AATAAAGGACTTCCATTCACTTGCAAGGCTTTTGTTTTTGATAAAGCAAGAAAACAATGC
     TTATTTCCTGAAGGTAAGTGAACGTTCCGAAAACAAAAACTATTTCGTTCTTTTGTTACG  1140
352  N  K  G  L  P  F  T  C  K  A  F  V  F  D  K  A  R  K  Q  C   371

1141 CTCTGGTTCCCCTTCAATAGCATGTCAAGTGGAGTGAAAAAAGAATTTGGCCATGAATTT
     GAGACCAAGGGGAAGTTATCGTACAGTTCACCTCACTTTTTTCTTAAACCGGTACTTAAA  1200
372  L  W  F  P  F  N  S  M  S  S  G  V  K  K  E  F  G  H  E  F   391
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(continued)

(continued)

```
1201 GACCTCTATGAAAACAAAGACTACATTAGAAACTGCATCATTGGTAAAGGACGCAGCTAC
     --------+---------+---------+---------+---------+---------+---------+
     CTGGAGATACTTTTGTTTCTGATGTAATCTTTGACGTAGTAACCATTTCCTGCGTCGATG 1260

 392 D  L  Y  E  N  K  D  Y  I  R  N  C  I  I  G  K  G  R  S  Y   411

1261 AAGGGAACAGTATCTATCACTAAGAGTGGCATCAAATGTCAGCCCTGGAGTTCCATGATA
     --------+---------+---------+---------+---------+---------+---------+
     TTCCCTTGTCATAGATAGTGATTCTCACCGTAGTTTACAGTCGGGACCTCAAGGTACTAT 1320

 412 K  G  T  V  S  I  T  K  S  G  I  K  C  Q  P  W  S  S  M  I   431

1321 CCACACGAACACAGCTATCGGGGTAAAGACCTACAGGAAAACTACTGTCGAAATCCTCGA
     --------+---------+---------+---------+---------+---------+---------+
     GGTGTGCTTGTGTCGATAGCCCCATTTCTGGATGTCCTTTTGATGACAGCTTTAGGAGCT 1380

 432 P  H  E  H  S  Y  R  G  K  D  L  Q  E  N  Y  C  R  N  P  R   451

1381 GGGGAAGAAGGGGGACCCTGGTGTTTCACAAGCAATCCAGAGGTACGCTACGAAGTCTGT
     --------+---------+---------+---------+---------+---------+---------+
     CCCCTTCTTCCCCCTGGGACCACAAAGTGTTCGTTAGGTCTCCATGCGATGCTTCAGACA 1440

 452 G  E  E  G  G  P  W  C  F  T  S  N  P  E  V  R  Y  E  V  C   471

1441 GACATTCCTCAGTGTTCAGAAGTTGAATGCATGACCTGCAATGGGGAGAGTTATCGAGGT
     --------+---------+---------+---------+---------+---------+---------+
     CTGTAAGGAGTCACAAGTCTTCAACTTACGTACTGGACGTTACCCCTCTCAATAGCTCCA 1500

 472 D  I  P  Q  C  S  E  V  E  C  M  T  C  N  G  E  S  Y  R  G  -   491

1501 CTCATGGATCATACAGAATCAGGCAAGATTTGTCAGCGCTGGGATCATCAGACACCACAC
     --------+---------+---------+---------+---------+---------+---------+
     GAGTACCTAGTATGTCTTAGTCCGTTCTAAACAGTCGCGACCCTAGTAGTCTGTGGTGTG 1560

 492 L  M  D  H  T  E  S  G  K  I  C  Q  R  W  D  H  Q  T  P  H   511

1561 CGGCACAAATTCTTGCCTGAAAGATATCCCGACAAGGGCTTTGATGATAATTATTGCCGC
     --------+---------+---------+---------+---------+---------+---------+
     GCCGTGTTTAAGAACGGACTTTCTATAGGGCTGTTCCCGAAACTACTATTAATAACGGCG 1620

 512 R  H  K  F  L  P  E  R  Y  P  D  K  G  F  D  D  N  Y  C  R   531

1621 AATCCCGATGGCCAGCCGAGGCCATGGTGCTATACTCTTGACCCTCACACCCGCTGGGAG
     --------+---------+---------+---------+---------+---------+---------+
     TTAGGGCTACCGGTCGGCTCCGGTACCACGATATGAGAACTGGGAGTGTGGGCGACCCTC 1680

 532 N  P  D  G  Q  P  R  P  W  C  Y  T  L  D  P  H  T  R  W  E   551

1681 TACTGTGCAATTAAAACATGCGCTGACAAAGCTGACGACGACGACAAACACCACCACCAC
     --------+---------+---------+---------+---------+---------+---------+
     ATGACACGTTAATTTTGTACGCGACTGTTTCGACTGCTGCTGCTGTTTGTGGTGGTGGTG 1740

 552 Y  C  A  I  K  T  C  A  D  K  A  D  D  D  D  K  H  H  H  H   571

1741 CACCACCACTAGGGTCGAC
     --------+--------- 1759
     GTGGTGGTGATCCCAGCTG

 572 H  H  H  *          574
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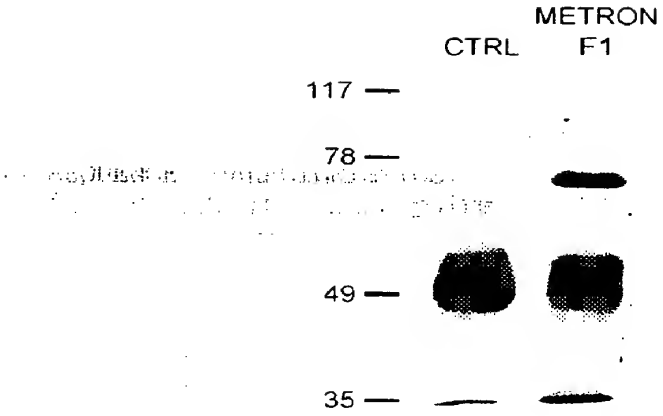


Fig 4

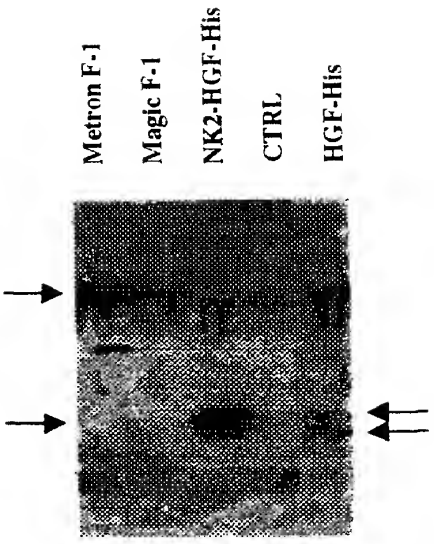


Fig 5A

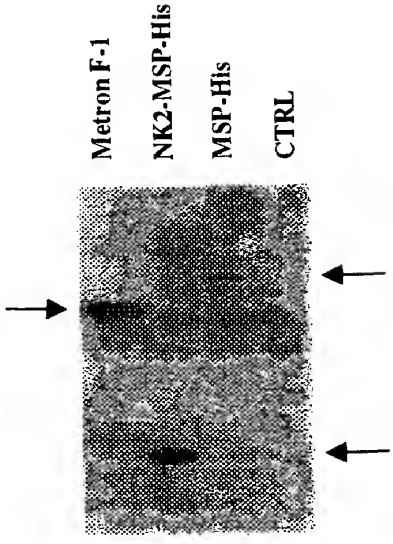


Fig 5B

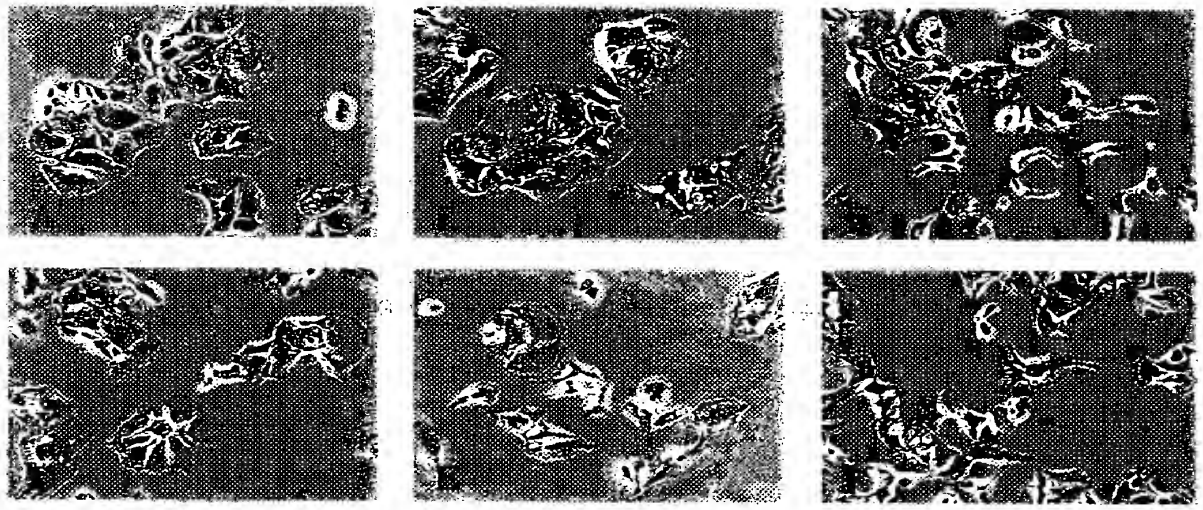


Fig 6

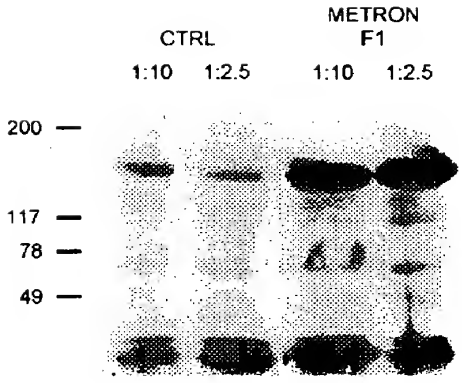
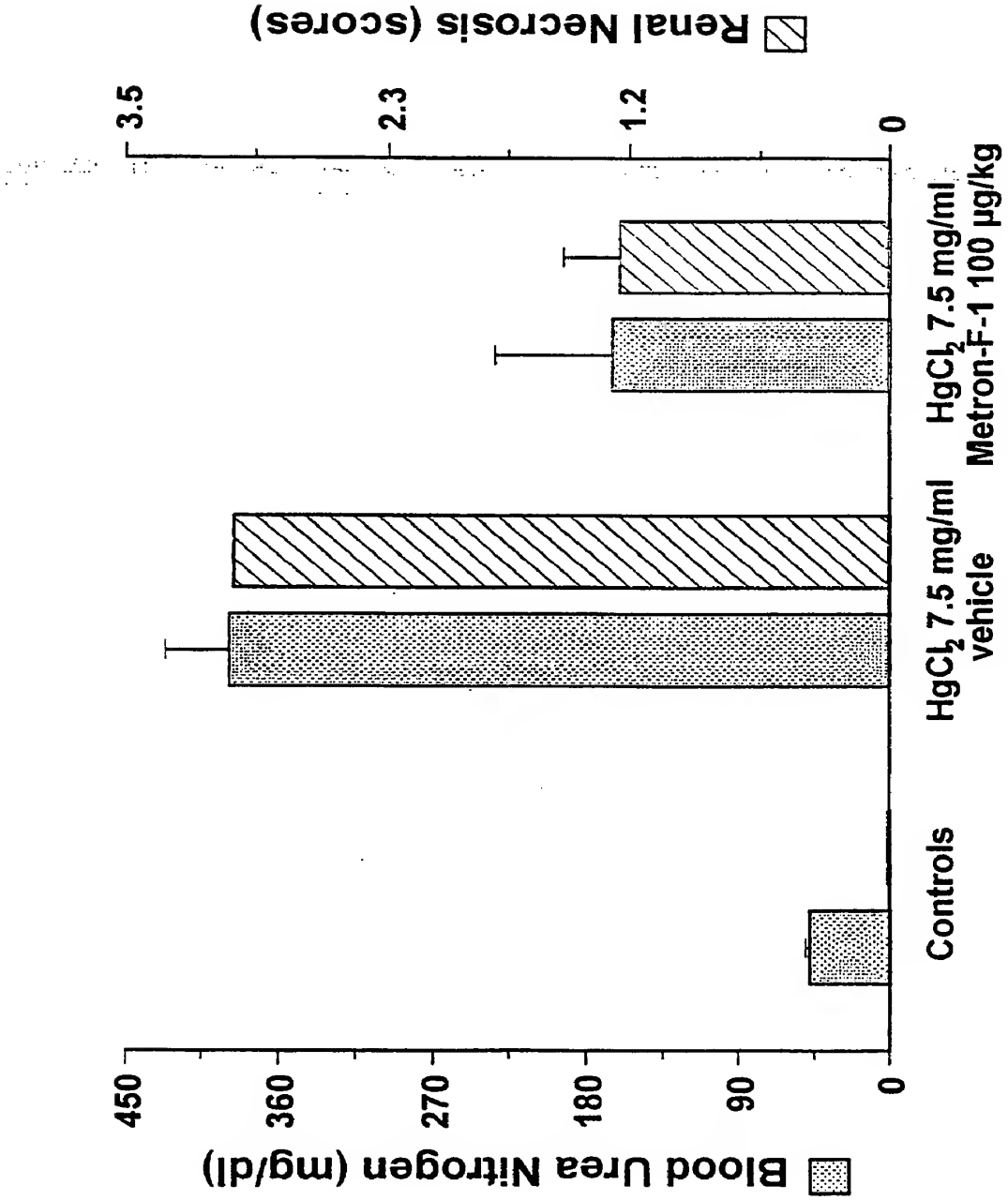


Fig 7

FIG 8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3.5
2.3
1.2
0
Blood Urea Nitrogen (mg/dl)
450
360
270
180
90
0
Controls
HgCl2 7.5 mg/ml vehicle
HgCl2 7.5 mg/ml Metron-F-1 100 µg/kg

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: DOMPE' S.p.A.
(B) STREET: Via Campo di Pile
(C) CITY: L'AQUILA
(E) COUNTRY: ITALY
(F) POSTAL CODE (ZIP): 67100

(ii) TITLE OF INVENTION: RECOMBINANT PROTEINS DERIVED FROM HGF AND MSP

(iii) NUMBER OF SEQUENCES: 4

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1725 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

ATGTGGGTGA CCAAACTCCT GCCAGCCCTG CTGCTGCAGC ATGTCCTCCT GCATCTCCTC 60

CTGCTCCCCA TCGCCATCCC CTATGCAGAG GGACAAAGGA AAAGAAGAAA TACAATTCAT 120

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GGCGGTTCTC TAGAGGGACA AAGGAAAAGA AGAAATACAA TTCATGAATT CAAAAAATCA 960

GCAAAGACTA CCCTAATCAA AATAGATCCA GCACTGAAGA TAAAAACCAA AAAAGTGAAT 1020

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GCTTTTGTTT TTGATAAAGC AAGAAAACAA TGCCTCTGGT TCCCCTTCAA TAGCATGTCA 1140

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ACAAGCAATC CAGAGGTACG CTACGAAGTC TGTGACATTC CTCAGTGTTC AGAAGTTGAA 1440

TGCATGACCT GCAATGGGGA GAGTTATCGA GGTCTCATGG ATCATACAGA ATCAGGCAAG 1500

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 574 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Arg Lys Arg Arg Asn Thr Ile His Glu Phe Lys Lys Ser Ala Lys Thr
        35                  40                  45

Thr Leu Ile Lys Ile Asp Pro Ala Leu Lys Ile Lys Thr Lys Lys Val
    50                  55                  60

Asn Thr Ala Asp Gln Cys Ala Asn Arg Cys Thr Arg Asn Lys Gly Leu
65                  70                  75                  80

Pro Phe Thr Cys Lys Ala Phe Val Phe Asp Lys Ala Arg Lys Gln Cys
                85                  90                  95

Leu Trp Phe Pro Phe Asn Ser Met Ser Ser Gly Val Lys Lys Glu Phe
            100                 105                 110

Gly His Glu Phe Asp Leu Tyr Glu Asn Lys Asp Tyr Ile Arg Asn Cys
        115                 120                 125

Ile Ile Gly Lys Gly Arg Ser Tyr Lys Gly Thr Val Ser Ile Thr Lys
    130                 135                 140

Ser Gly Ile Lys Cys Gln Pro Trp Ser Ser Met Ile Pro His Glu His
145                 150                 155                 160

Ser Tyr Arg Gly Lys Asp Leu Gln Glu Asn Tyr Cys Arg Asn Pro Arg
                165                 170                 175

Gly Glu Glu Gly Gly Pro Trp Cys Phe Thr Ser Asn Pro Glu Val Arg
            180                 185                 190

Tyr Glu Val Cys Asp Ile Pro Gln Cys Ser Glu Val Glu Cys Met Thr
        195                 200                 205

Cys Asn Gly Glu Ser Tyr Arg Gly Leu Met Asp His Thr Glu Ser Gly
    210                 215                 220
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Lys Ile Cys Gln Arg Trp Asp His Gln Thr Pro His Arg His Lys Phe
225 230 235 240

Leu Pro Glu Arg Tyr Pro Asp Lys Gly Phe Asp Asp Asn Tyr Cys Arg
245 250 255

Asn Pro Asp Gly Gln Pro Arg Pro Trp Cys Tyr Thr Leu Asp Pro His
260 265 270

Thr Arg Trp Glu Tyr Cys Ala Ile Lys Thr Cys Ala Asp Lys Ala Ser
275 280 285

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Leu
290 295 300

Glu Gly Gln Arg Lys Arg Arg Asn Thr Ile His Glu Phe Lys Lys Ser
305 310 315 320

Ala Lys Thr Thr Leu Ile Lys Ile Asp Pro Ala Leu Lys Ile Lys Thr
325 330 335

Lys Lys Val Asn Thr Ala Asp Gln Cys Ala Asn Arg Cys Thr Arg Asn
340 345 350

Lys Gly Leu Pro Phe Thr Cys Lys Ala Phe Val Phe Asp Lys Ala Arg
355 360 365

Lys Gln Cys Leu Trp Phe Pro Phe Asn Ser Met Ser Ser Gly Val Lys
370 375 380

Lys Glu Phe Gly His Glu Phe Asp Leu Tyr Glu Asn Lys Asp Tyr Ile
385 390 395 400

Arg Asn Cys Ile Ile Gly Lys Gly Arg Ser Tyr Lys Gly Thr Val Ser
405 410 415

Ile Thr Lys Ser Gly Ile Lys Cys Gln Pro Trp Ser Ser Met Ile Pro
420 425 430

His Glu His Ser Tyr Arg Gly Lys Asp Leu Gln Glu Asn Tyr Cys Arg
435 440 445

Asn Pro Arg Gly Glu Glu Gly Gly Pro Trp Cys Phe Thr Ser Asn Pro
450 455 460

Glu Val Arg Tyr Glu Val Cys Asp Ile Pro Gln Cys Ser Glu Val Glu
465 470 475 480

Cys Met Thr Cys Asn Gly Glu Ser Tyr Arg Gly Leu Met Asp His Thr
485 490 495

Glu Ser Gly Lys Ile Cys Gln Arg Trp Asp His Gln Thr Pro His Arg
500 505 510

His Lys Phe Leu Pro Glu Arg Tyr Pro Asp Lys Gly Phe Asp Asp Asn
515 520 525

Tyr Cys Arg Asn Pro Asp Gly Gln Pro Arg Pro Trp Cys Tyr Thr Leu
530 535 540

Asp Pro His Thr Arg Trp Glu Tyr Cys Ala Ile Lys Thr Cys Ala Asp
545 550 555 560

Lys Ala Asp Asp Asp Asp Lys His His His His His His His
565 570

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1692 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

ATGGGGTGGC TCCCACTCCT GCTGCTTCTG ACTCAATGCT TAGGGGTCCC TGGGCAGCGC 60

TCGCCATTGA ATGACTTCCA AGTGCTCCGG GGCACAGAGC TACAGCACCT GCTACATGCG 120

GTGGTGCCCG GGCCTTGGCA GGAGGATGTG GCAGATGCTG AAGAGTGTGC TGGTCGCTGT 180

GGGCCCTTAA TGGACTGCCG GGCCTTCCAC TACAACGTGA GCAGCCATGG TTGCCAACTG 240

CTGCCATGGA CTCAACACTC GCCCCACACG AGGCTGCGGC GTTCTGGGCG CTGTGACCTC 300

TTCCAGAAGA AAGACTACGT ACGGACCTGC ATCATGAACA ATGGGGTTGG GTACCGGGGC 360

ACCATGGCCA CGACCGTGGG TGGCCTGCCC TGCCAGGCTT GGAGCCACAA GTTCCCGAAT 420

GATCACAAGT ACACGCCCAC TCTCCGGAAT GGCCTGGAAG AGAACTTCTG CCGTAACCCT 480

GATGGCGACC CCGGAGGTCC TTGGTGCTAC ACAACAGACC CTGCTGTGCG CTTCCAGAGC 540

TGCGGCATCA AATCCTGCCG GGAGGCCGCG TGTGTCTGGT GCAATGGCGA GGAATACCGC 600

GGCGCGGTAG ACCGCACGGA GTCAGGGCGC GAGTGCCAGC GCTGGGATCT TCAGCACCCG 660

CACCAGCACC CCTTCGAGCC GGGCAAGTTC CTCGACCAAG GTCTGGACGA CAACTATTGC 720

CGGAATCCTG ACGGCTCCGA GCGGCCATGG TGCTACACTA CGGATCCGCA GATCGAGCGA 780

GAGTTCTGTG ACCTCCCCCG CTGCGGGTCC GAGGCACAGC CCCGCCTCGA GGGCGGTGGC 840

GGTTCTGGTG GCGGTGGCTC CGGCGGTGGC GGTTCTCTAG AGGGACAAAG GAAAAGAAGA 900

AATACAATTC ATGAATTCAA AAAATCAGCA AAGACTACCC TAATCAAAAT AGATCCAGCA 960

CTGAAGATAA AAACCAAAAA AGTGAATACT GCAGACCAAT GTGCTAATAG ATGTACTAGG 1020

AATAAAGGAC TTCCATTCAC TTGCAAGGCT TTTGTTTTTG ATAAAGCAAG AAAACAATGC 1080

CTCTGGTTCC CCTTCAATAG CATGTCAAGT GGAGTGAAAA AAGAATTTGG CCATGAATTT 1140

GACCTCTATG AAAACAAAGA CTACATTAGA AACTGCATCA TTGGTAAAGG ACGCAGCTAC 1200

AAGGGAACAG TATCTATCAC TAAGAGTGGC ATCAAATGTC AGCCCTGGAG TTCCATGATA 1260

CCACACGAAC ACAGCTATCG GGGTAAAGAC CTACAGGAAA ACTACTGTCG AAATCCTCGA 1320

GGGGAAGAAG GGGGACCCTG GTGTTTCACA AGCAATCCAG AGGTACGCTA CGAAGTCTGT 1380

GACATTCCTC AGTGTTCAGA AGTTGAATGC ATGACCTGCA ATGGGGAGAG TTATCGAGGT 1440

CTCATGGATC ATACAGAATC AGGCAAGATT TGTCAGCGCT GGGATCATCA GACACCACAC 1500

CGGCACAAAT TCTTGCCTGA AAGATATCCC GACAAGGGCT TTGATGATAA TTATTGCCGC 1560

AATCCCGATG GCCAGCCGAG GCCATGGTGC TATACTCTTG ACCCTCACAC CCGCTGGGAG 1620

TACTGTGCAA TTAAAACATG CGCTGACAAA GCTGACGACG ACGACAAACA CCACCACCAC 1680

CACCACCACT AG 1692

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 563 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Gly Trp Leu Pro Leu Leu Leu Leu Thr Gln Cys Leu Gly Val
1 5 10 15

Pro Gly Gln Arg Ser Pro Leu Asn Asp Phe Gln Val Leu Arg Gly Thr
20 25 30

Glu Leu Gln His Leu Leu His Ala Val Val Pro Gly Pro Trp Gln Glu
35 40 45

Asp Val Ala Asp Ala Glu Glu Cys Ala Gly Arg Cys Gly Pro Leu Met
50 55 60

Asp Cys Arg Ala Phe His Tyr Asn Val Ser Ser His Gly Cys Gln Leu
65 70 75 80

Leu Pro Trp Thr Gln His Ser Pro His Thr Arg Leu Arg Arg Ser Gly
85 90 95

Arg Cys Asp Leu Phe Gln Lys Lys Asp Tyr Val Arg Thr Cys Ile Met
100 105 110

Asn Asn Gly Val Gly Tyr Arg Gly Thr Met Ala Thr Thr Val Gly Gly
115 120 125

Leu Pro Cys Gln Ala Trp Ser His Lys Phe Pro Asn Asp His Lys Tyr
130 135 140

Thr Pro Thr Leu Arg Asn Gly Leu Glu Glu Asn Phe Cys Arg Asn Pro
145 150 155 160

Asp Gly Asp Pro Gly Gly Pro Trp Cys Tyr Thr Thr Asp Pro Ala Val
165 170 175

Arg Phe Gln Ser Cys Gly Ile Lys Ser Cys Arg Glu Ala Ala Cys Val
180 185 190

Trp Cys Asn Gly Glu Glu Tyr Arg Gly Ala Val Asp Arg Thr Glu Ser
195 200 205

Gly Arg Glu Cys Gln Arg Trp Asp Leu Gln His Pro His Gln His Pro
210 215 220

Phe Glu Pro Gly Lys Phe Leu Asp Gln Gly Leu Asp Asp Asn Tyr Cys
225 230 235 240

Arg Asn Pro Asp Gly Ser Glu Arg Pro Trp Cys Tyr Thr Thr Asp Pro
245 250 255

Gln Ile Glu Arg Glu Phe Cys Asp Leu Pro Arg Cys Gly Ser Glu Ala
260 265 270

Gln Pro Arg Leu Glu Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
275 280 285

Gly Gly Gly Ser Leu Glu Gly Gln Arg Lys Arg Arg Asn Thr Ile His
290 295 300

Glu Phe Lys Lys Ser Ala Lys Thr Thr Leu Ile Lys Ile Asp Pro Ala
305 310 315 320

Leu Lys Ile Lys Thr Lys Lys Val Asn Thr Ala Asp Gln Cys Ala Asn
325 330 335

Arg Cys Thr Arg Asn Lys Gly Leu Pro Phe Thr Cys Lys Ala Phe Val
340 345 350

Phe Asp Lys Ala Arg Lys Gln Cys Leu Trp Phe Pro Phe Asn Ser Met
355 360 365

Ser Ser Gly Val Lys Lys Glu Phe Gly His Glu Phe Asp Leu Tyr Glu
370 375 380

Asn Lys Asp Tyr Ile Arg Asn Cys Ile Ile Gly Lys Gly Arg Ser Tyr
385 390 395 400

Lys Gly Thr Val Ser Ile Thr Lys Ser Gly Ile Lys Cys Gln Pro Trp
405 410 415

Ser Ser Met Ile Pro His Glu His Ser Tyr Arg Gly Lys Asp Leu Gln
420 425 430

Glu Asn Tyr Cys Arg Asn Pro Arg Gly Glu Glu Gly Gly Pro Trp Cys
435 440 445

Phe Thr Ser Asn Pro Glu Val Arg Tyr Glu Val Cys Asp Ile Pro Gln
450 455 460

Cys Ser Glu Val Glu Cys Met Thr Cys Asn Gly Glu Ser Tyr Arg Gly
465 470 475 480

Leu Met Asp His Thr Glu Ser Gly Lys Ile Cys Gln Arg Trp Asp His
485 490 495

Gln Thr Pro His Arg His Lys Phe Leu Pro Glu Arg Tyr Pro Asp Lys
500 505 510

Gly Phe Asp Asp Asn Tyr Cys Arg Asn Pro Asp Gly Gln Pro Arg Pro
515 520 525

Trp Cys Tyr Thr Leu Asp Pro His Thr Arg Trp Glu Tyr Cys Ala Ile
530 535 540

Lys Thr Cys Ala Asp Lys Ala Asp Asp Asp Asp Lys His His His His
545 550 555 560

His His His